# THE
# JOURNAL OF PHYSIOLOGY

EDITED BY

J. N. LANGLEY, Sc.D., F.R.S.

AIDED, IN THE SELECTION OF PAPERS FOR PUBLICATION, BY

Prof. W. M. BAYLISS (London).
Sir DAVID FERRIER (London).
Dr J. S. HALDANE (Oxford).
Prof. W. D. HALLIBURTON (London).
Mr W. B. HARDY (Cambridge).
Prof. F. G. HOPKINS (Cambridge).
Prof. A. B. MACALLUM (Toronto).
Prof. D. NOËL PATON (Glasgow).
Prof. W. A. OSBORNE (Melbourne).
Prof. C. S. SHERRINGTON (Oxford).
Prof. E. H. STARLING (London).
Prof. W. STIRLING (Manchester).
Sir T. ANDERSON STUART (Sydney).
Prof. A. WALLER (London).

Vol. LII.

1918—1919

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C. 4.

13
126
R67

# CONTENTS OF VOL. LII.

*No.* 1. *April* 16, 1918.

PAGE

The guanidin content of muscle in tetania parathyreopriva. By **Pearl S. Henderson** . . . . . . . . . . . . 1

The velocity factor in cardiac work. By **C. Lovatt Evans** . . 6

Observations on the atrophy of denervated muscle. By **J. N. Langley** and **M. Hashimoto** . . . . . . . . . 15

On the creatin content of skeletal muscle in degeneration following denervation. By **E. P. Cathcart**, **P. S. Henderson** and **D. Noël Paton** . . . . . . . . . . . . . . 70

*Nos.* 2 & 3. *July* 10, 1918.

On the possibility of mixing the air in the lungs with foreign air, especially as it is used in Krogh and Lindhard's nitrous oxide method. By **Carl Sonne** . . . . . . . . . . . . . . 75

The effect of thyro-parathyroidectomy on the heart and circulation. By **David Burns** and **Alexander McL. Watson** . . . . . 88

The nutritive value of certain fish. By **J. C. Drummond** . . 95

The vasodilator action of histamine and of some other substances. By **H. H. Dale** and **A. N. Richards** . . . . . . . . 110

The distribution of blood, glycogen and fat in the lobes of the liver. By **V. B. Dowler** and **V. H. Mottram** . . . . . . . 166

*No.* 4. *October* 18, 1918.

Chromatic aberration and resolving power of the eye. By **H. Hartridge** . . . . . . . . . . . . . 175

On the stimulation and paralysis of nerve cells and nerve endings. Part II. Paralysis by curari, strychnine and brucine and its antagonism by nicotine. By **J. N. Langley** . . . . . . . 247

## *No.* 5. *March* 25, 1919.

PAGE

The control of the physical regulation of the body temperature. By J. M. O'Connor . . . . . . . . . . . . . 267

The spectrocomparator, an apparatus designed for the determination of the percentage saturation of blood with oxygen or carbon monoxide. By August Krogh . . . . . . . . . 281

The respiratory function of the blood in fishes. By A. Krogh and I. Leitch . . . . . . . . . . . . . . . 288

Adrenalin and pituitrin—a study in interaction and interrelation. By Douglas Cow . . . . . . . . . . . . 301

Some further observations on blood reaction. By J. F. Donegan and T. R. Parsons . . . . . . . . . . . . 315

Fats and fatty acids as food. By a Committee of the Royal Society . . . . . . . . . . . . . . . 328

A contribution to the physiology of the aqueous humour. By W. A. Osborne . . . . . . . . . . . . . . . 347

A simple method of short-circuiting the portal circulation. By H. H. Dale and P. P. Laidlaw . . . . . . . . . . . 351

Histamine shock. By H. H. Dale and P. P. Laidlaw . . . . 355

## *No.* 6. *May* 20, 1919.

The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. By August Krogh . . 391

The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. By August Krogh . . . . . . . . . . . . . 409

Influence of saprophyte bacteria on oxidation in higher animals. (5th series of experiments.) By I. Kianizin . . . . . . 416

The respiratory response to anoxæmia. By J. S. Haldane, J. C. Meakins and J. G. Priestley . . . . . . . . . . 420

The effects of shallow breathing. By J. S. Haldane, J. C. Meakins and J. G. Priestley . . . . . . . . . . . . . 433

The physiological action of extracts of the electrical organs of the skate (*Raia clavata*) and torpedo (*T. marmorata*). By P. T. Herring . 454

The supply of oxygen to the tissues and the regulation of the capillary circulation. By August Krogh . . . . . . 457

## LIST OF AUTHORS.

PAGE
Burns, D. and Watson, A. McL. Thyro-parathyroidectomy . . . 88
Burns, D., Halliburton, W. D., Paton, D. Noël and Sharpe, J. S. Fats as food . . . 328
Cathcart, E. P., Henderson, P. S. and Paton, D. Noël. Creatin of muscle . . . 70
Committee of the Royal Society. Fats as food . . . 328
Cow, D. Adrenalin and pituitrin . . . 301
Dale, H. H. and Richards, A. N. Histamine vasodilation . . . 110
Dale, H. H. and Laidlaw, P. P. Short circuit of portal blood . . . 351
Dale, H. H. and Laidlaw, P. P. Histamine shock . . . 355
Donegan, J. F. and Parsons, T. R. Reaction of blood . . . 315
Dowler, V. B. and Mottram, V. H. Variation in liver lobes . . . 166
Drummond, J. C. Nutritive value of fish . . . 95
Evans, C. Lovatt. Work of heart . . . 6
Haldane, J. S., Meakins, J. C. and Priestley, J. G. Anoxæmia . . . 420
Haldane, J. S., Meakins, J. C. and Priestley, J. G. Shallow breathing . . . 433
Halliburton, W. D., Burns, D., Paton, D. Noël and Sharpe, J. S. Fats as food . . . 328
Hartridge, H. Chromatic aberration . . . 175
Hashimoto, M. and Langley, J. N. Denervated muscle atrophy . . . 15
Henderson, P. S. Guanidin and parathyroids . . . 1
Henderson, P. S., Paton, D. Noël and Cathcart, E. P. Creatin of muscle . . . 70
Herring, P. T. Electrical organs . . . 454
Kianizin, I. Bacteria and oxidation . . . 416
Krogh, A. Spectrocomparator . . . 281
Krogh, A. and Leitch, I. Fish blood respiration . . . 288
Krogh, A. Gases through tissues . . . 391
Krogh, A. Capillaries and oxygen in muscle . . . 409
Krogh, A. Capillary circulation . . . 457
Laidlaw, P. P. and Dale, H. H. Short circuit of portal blood . . . 351
Laidlaw, P. P. and Dale, H. H. Histamine shock . . . 355
Langley, J. N. and Hashimoto, M. Denervated muscle atrophy . . . 15
Langley, J. N. Peripheral nerve-cell paralysis . . . 247
Leitch, I. and Krogh, A. Fish blood respiration . . . 288
Meakins, J. C., Priestley, J. G. and Haldane, J. S. Anoxæmia . . . 420
Meakins, J. C., Priestley, J. G. and Haldane, J. S. Shallow breathing . . . 433
Mottram, V. H. and Dowler, V. B. Variation in liver lobes . . . 166
O'Connor, J. M. Body temperature regulation . . . 267

PAGE
**Osborne**, W. A. Aqueous humour . . . . . . . . 347
**Parsons**, T. R. and **Donegan**, J. F. Reaction of blood . . . 315
**Paton**, D. Noël, **Burns**, D., **Halliburton**, W. D. and **Sharpe**, J. S. Fats as food . . . . . . . . . . . 328
**Paton**, D. Noël, **Cathcart**, E. P. and **Henderson**, P. S. Creatin of muscle . . . . . . . . . . . . . 70
**Priestley**, J. G., **Haldane**, J. S. and **Meakins**, J. C. Anoxæmia . 420
**Priestley**, J. G., **Haldane**, J. S. and **Meakins**, J. C. Shallow breathing . . . . . . . . . . . . 433
**Richards**, A. N. and **Dale**, H. H. Histamine vasodilation . . 110
**Sharpe**, J. S., **Burns**, D., **Halliburton**, W. D. and **Paton**, D. Noël. Fats as food . . . . . . . . . . . 328
**Sonne**, Carl. Air mixing in lungs . . . . . . . . 75
**Watson**, A. McL. and **Burns**, D. Thyro-parathyroidectomy . . 88

## PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY.

*July* 28, 1917.

*Hartridge, H. and Winfield, G.* A high speed shaking machine . . . i
*Atkinson, Mildred.* The histological structure of the moderator band of the sheep . . . . . . . . . . . . . iii

*December* 8, 1917.

*Waller, A. D.* Demonstration of the psycho-galvanic phenomenon . . . vi

*January* 26, 1918.

*Cramer, W.* Further observations on the thyroid-adrenal apparatus. A histochemical method for the demonstration of adrenalin granules in the suprarenal gland . . . . . . . . . . viii
*Mellanby, Edward.* The part played by an "accessory factor" in the production of experimental rickets . . . . . . . . xi

*March* 16, 1918.

*Cramer, W.* Histochemical observations on the functional activity of the suprarenal medulla in different pathological conditions . . . xiii
*Buckmaster, George A.* The transport of carbon dioxide by solutions of sodium bicarbonate . . . . . . . . . . . . xvi
*Bayliss, W. M.* Gum injections in "shock" . . . . . . . xvii
*Mottram, V. H.* Sudan III and the detection of fat . . . . . . xviii
*Waller, A. D.* The lost time of the emotive response of the human subject . xxi
*Waller, A. D.* The alteration of conductivity produced in the pad of the cat's foot (post mortem) by excitation of the sciatic nerve . . . xxiii

*May* 18, 1918.

*Langley, J. N.* Histological changes in nerve after compression without rupture of sheaths (*Preliminary communication*) . . . . xxvi

*June* 29, 1918.

PAGE
*Gunn, J. A.* A simple respiratory stethograph . . . . . . . xxix
*Homer, Annie.* On the concentration of antitoxic sera . . . . . xxxi
*Homer, Annie.* A note on the nature of the proteins associated with antitoxin in antitoxic sera . . . . . . . . . . . . . xxxiii
*Homer, Annie.* On the toxicity of cresylic acid . . . . . . . . xxxiv
*Symes, W. L.* Note on the survival of the heart of the stale summer frog . xxxv
*Homer, Annie.* Further observations on the properties of antitoxic sera . xxxvii
*Burns, David.* A note on the influence of guanidin on the anaphylactic state. (*Preliminary communication*) . . . . . . . . . . xxxix

*October* 19, 1918.

*Henderson, P. S.* On the creatin content of the muscles in rickets in dogs . xli

*December* 14, 1918.

*Langley, J. N.* Persistence of the central somatic effect of strychnine after a large dose of nicotine . . . . . . . . . . . . xliv
*Bullock, W. E. and Cramer, W.* On a new accessory factor in bacterial infections. (*Preliminary communication*) . . . . . . xlv
*Symes, W. L.* A student's apparatus for perfusion of the suspended frog's heart . . . . . . . . . . . . . . . xlvi
*Waller, A. D.* Calibration of a dock labourer by means of his $CO_2$ discharge xlviii
*Waller, A. D.* The cost of mechanical work in terms of $CO_2$ expired . . l
*Symes, W. L.* A recording siphon . . . . . . . . . lii
*Mellanby, Edward.* A further demonstration of the part played by accessory food factors in the ætiology of rickets . . . . . . . . liii
*Jona, Judah Leon.* A note in reference to segmental distribution of blood-vessels in the upper limb . . . . . . . . . . liv

*January* 25, 1919.

*Da Fano, C.* Modifications of Bielschowsky's method . . . . . lvii
*Waller, A. D.* The $CO_2$ ordinate of a dock-labourer (Tom King) during six days' work. (Winter hours 7.30 a.m. to 12; 1 p.m. to 4.30) . . lix
*Bazett, H. C.* A mechanical pipette for easy measurement of small volumes lxi
*Kellaway, C. H.* Some physiological effects of anoxæmia . . . . . lxiii
*Bainbridge, F. A. and Hilton, R.* The relation between respiration and the pulse rate . . . . . . . . . . . . . . lxv

*February* 22, 1919.

*Waller, A. D.* The time-relation between muscular work and $CO_2$ discharge lxvii
*Hill, Leonard and Hargood-Ash, D.* Metabolism and cooling power . . xlix

*March* 22, 1919.

*Waller, A. D.* "Work" measured by $CO_2$ . . . . . . . . . lxxii
*Krogh, August.* Energy exchange in man . . . . . . . . lxxiv

# LIST OF AUTHORS.

PAGE
Atkinson, Mildred. Structure of the moderator band of the sheep . . iii
Bainbridge, F. A. and Hilton, R. Respiration and pulse. . . . . lxv
Bayliss, W. M. Gum injections in "shock" . . . . . . . . xvii
Bazett, H. C. Mechanical pipette for easy measurement of small volumes . lxi
Buckmaster, George A. Transport of carbon dioxide . . . . . xvi
Bullock, W. E. and Cramer, W. Accessory factor in bacterial infections . xlv
Burns, David. Influence of guanidin on the anaphylactic state . . xxxix
Cramer, W. Thyroid-adrenal apparatus . . . . . . . . viii
Cramer, W. Functional activity of suprarenal medulla . . . . . xiii
Cramer, W. and Bullock, W. E. Accessory factor in bacterial infections . xlv
Da Fano, C. Modifications of Bielschowsky's method . . . . lvii
Gunn, J. A. A simple respiratory stethograph . . . . . . . xxix
Hargood-Ash, D. and Hill Leonard. Metabolism and cooling power . lxix
Hartridge, H. and Winfield, G. High speed shaking machine . . i
Henderson, P. S. Creatin content of the muscles in rickets in dogs . . xli
Hill, Leonard and Hargood-Ash, D. Metabolism and cooling power . lxix
Hilton, R. and Bainbridge, F. A. Respiration and pulse . . . . . lxv
Homer, Annie. Concentration of antitoxic sera . . . . . . . xxxi
Homer, Annie. Proteins associated with antitoxin in antitoxic sera . . xxxiii
Homer, Annie. Toxicity of cresylic acid . . . . . . . . xxxiv
Homer, Annie. Properties of antitoxic sera . . . . . . . xxxvii
Jona, Judah Leon. Segmental distribution of blood-vessels . . . liv
Kellaway, C. H. Physiological effects of anoxæmia . . . . . lxiii
Krogh, August. Energy exchange in man . . . . . . . lxxiv
Langley, J. N. Changes in nerve after compression . . . . . . xxvi
Langley, J. N. Central somatic effect of strychnine . . . . . . xliv
Mellanby, Edward. "Accessory factor" in experimental rickets . . xi
Mellanby, Edward. Part played by accessory food factors . . . liii
Mottram, V. H. Sudan III and the detection of fat . . . . . . xviii
Symes, W. L. Survival of the heart of the stale summer frog . . . xxxv
Symes, W. L. Apparatus for perfusion of the suspended frog's heart . . xlvi
Symes, W. L. A recording siphon . . . . . . . . . . lii
Waller, A. D. Psycho-galvanic phenomenon . . . . . . . vi
Waller, A. D. Lost time of the emotive response . . . . . . xxi
Waller, A. D. Alteration of conductivity in the pad of the cat's foot . xxiii
Waller, A. D. Calibration of a dock labourer . . . . . . . xlviii
Waller, A. D. Cost of mechanical work in terms of $CO_2$ expired . . l
Waller, A. D. $CO_2$ ordinate of a dock-labourer during six days work . lix
Waller, A. D. Time-relation between muscular work and $CO_2$ discharge . lxvii
Waller, A. D. "Work" measured by $CO_2$ . . . . . . . . lxxii
Winfield, G. and Hartridge, H. High speed shaking machine . . i

# THE GUANIDIN CONTENT OF MUSCLE IN TETANIA PARATHYREOPRIVA. BY PEARL S. HENDERSON.

*(From the Physiological Laboratory, University of Glasgow.)*

THE investigations of Noël Paton, Findlay and their co-workers(1) have correlated the symptoms of tetania parathyreopriva with the action of guanidin on the motor cells of the spinal cord. Burns and Sharpe(2) have shown that in this condition there is an increase of guanidin in the blood and in the urine. So far no work has been recorded which throws light upon the possible source of this guanidin. It seemed possible that it might come either from an increased formation of the substance in the muscles, or from an increased liberation in some altered metabolism of muscle. It therefore seemed desirable to determine whether the amount of guanidin in muscle is modified after ablation of the parathyroids. Since creatin is methyl guanidin acetic acid, and since its amount has been correlated with the tone of muscle by Pekelharing(3) and others, it appeared to be of some interest to investigate variations in its amount.

In the first series of experiments the total guanidin was determined. This included the free guanidin of muscle, the creatin guanidin, and guanidin in such complexes as arginin. In the second series of experiments an attempt was made to determine the free guanidin content. In both series the amount of creatin present was investigated.

### *Total guanidin.*

The experiments were performed on dogs, and the muscle used was the gastrocnemius. 10 gms. of muscle, from which, before weighing, all visible traces of fatty tissue had been removed, were cut into small pieces and hydrolysed with 160 c.c. of 25 p.c. sulphuric acid for 8 to 10 hours until the whole had gone into solution. The method of estimating the guanidin present in the solution was that described by Burns(4), 20 gms. of calcium permanganate being found sufficient for the oxidation. When the first crop of crystals of guanidin picrate had been removed, a second crop was usually obtained after the filtrate had stood for one or two days, and in some cases a third lot was formed. In these cases care had to be taken that the substance thus obtained was not sodium picrate. Microscopic examination of the crystals in each case confirmed their identity. The whole analysis takes from 6–7 days and the number of observations had therefore to be limited.

TABLE A.

| | Parathyroidectomised | | Normal |
|---|---|---|---|
| I. | 0·387 % | VI. | 0·560 % |
| II. | ·416 | VII. | ·520 |
| III. | ·392 | VIII. | ·540 |
| IV. | ·385 | IX. | ·580 |
| V. | ·378 | X. | ·629 |
| Average | 0·391 ± ·025 | Average | 0·565 ± ·064 |

The creatin was estimated as creatinin as described by Brown and Cathcart(5). The estimations were made on 5 gms. of muscle when possible, on 2 gms. when small dogs were used and the gastrocnemius was not very large.

TABLE B.

| | Parathyroidectomised | | Normal |
|---|---|---|---|
| I. | 0·25 %* | VI. | 0·237 %* |
| II. | ·30 | VII. | ·225 |
| IV | ·25 | VIII. | ·238 |
| V. | ·27 | | |
| Average | ·267 ± ·033 | Average | ·231 ± ·007 |

(see also Tables D and E.)

* Work done by Miss Andross.

The results of the work so far may be summarised for comparison as in the appended table.

TABLE C.

| | Results of Parathyroidectomy upon | | |
|---|---|---|---|
| | Guanidin | Creatin | Creatinin |
| Muscle | − | + | |
| Blood | + | | |
| Urine | + | d | − |

(+ increase; − decrease; d, presence doubtful.)

The increased creatin content of muscle may be due to a retention of the creatin, or some precursor of creatin, in the muscle. It may indicate an attempt on the part of the muscle to methylate and thus render harmless some portion of the guanidin which is liberated and is being swept away.

The work done by **Burns** and **Sharpe** (*op. cit.*) on the presence of guanidin in the blood and the urine after parathyroidectomy considered along with the decrease in muscle content found here suggests that a liberation of the guanidin occurs, that it passes to the blood to act upon the nervous system, and then escapes in the urine. But on the other hand, the increased amount of guanidin in the blood and urine, with a decrease in the amount in muscle, may indicate that parathyroidectomy brings about the destruction of some power, possessed by the normal muscle, of taking up guanidin from the blood, in which case the amount of free guanidin in muscle after parathyroidectomy would also be modified. It therefore seemed possible that investigation of the variations of the free guanidin content might throw some light on this, for while the increase in guanidin in creatin is opposed to the view, a decrease in the free guanidin would rather support it.

### *Free guanidin.*

These experiments were performed upon cats—5 grms. of gastrocnemius were cut into very small pieces and rubbed up with clean, freshly washed sand, water being added gradually. This mixture was transferred to an Erlenmeyer flask, a few drops of chloroform and thymol solution added, and then placed on the top of a hot water oven of inside temperature 52° C., and left there for 14–16 hours. At the end of this time the solution was filtered, the residue being thoroughly washed. The method employed was that described by **Koch**(6) to isolate the guanidin as methyl guanidin aurochloride, modified by **Sharpe** in his work on the guanidin content of blood. A trial investigation was made and the crystals of the aurochloride finally obtained were so clean and so perfectly characteristic that the use of mercuric chloride to remove the bases, which is such a tedious process, seemed unnecessary.

The creatin content was estimated, as previously described, in the case of six out of the nine animals used.

TABLE D. Free guanidin and creatin.

| | Parathyroidectomised | | | Normal | |
|---|---|---|---|---|---|
| No. | Guanidin | Creatin | No. | Guanidin | Creatin |
| I. | 0·0569 % | — | II.[*] | 0·0760 % | — |
| III. | ·0426 | 0·290 % | VI. | ·0895 | 0·230 % |
| IV. | ·0513 | ·310 | VII. | ·0907 | ·246 |
| V. | ·0472 | ·271 | VIII. | ·0776 | ·220 |
| IX. | ·0411 | — | | | |
| Average | ·0517 ± ·0052 | ·290 ± ·02 | Average | ·0839 ± ·0068 | ·232 ± ·014 |

---

[*] Cat (II) was operated upon on September 19th—complete parathyroidectomy being

A distinct decrease in the free guanidin is indicated, while the increase in the creatin noted in the previous experiments is confirmed.

Muscle contains other substances besides those containing nitrogen—*e.g.* water, fats, glycogen, etc., and the possibility of the variations in the percentage of guanidin being due to changes in those, had to be considered. The fact that the creatin is increased while the guanidin is decreased seems to indicate that the changes are not due to mere alterations in the proportion of the non-nitrogenous constituents. To settle this point the variations in the total nitrogen of the muscle were determined in a series of cats, normal and suffering from tetania parathyreopriva. The creatin content of the muscle was determined at the same time.

TABLE E.

| | Parathyroidectomised | | | Normal | |
|---|---|---|---|---|---|
| Cat | Nitrogen | Creatin | Cat | Nitrogen | Creatin |
| I. | 3·5 % | 0·322 % | II. | 4·10 % | 0·318 % |
| III. | 2·66 | ·382 | IV. | 3·50 | ·201 |
| V. | 3·78 | ·345 | VI. | 3·64 | ·244 |
| VIII. | 2·80 | ·360 | VII. | 3·92 | — |
| Average | 3·185 | ·352 | Average | 3·79 | ·284 |

This shows a fall of 16 p.c. in the total nitrogen. But since the fall in the total guanidin was found to be fully 30 p.c. and the fall in free guanidin 38 p.c.—there is a definite fall in relationship to the nitrogenous constituents of the muscle.

When the guanidin and creatin nitrogen throughout the whole series is calculated as percentage of the total, the following results are obtained:

TABLE F.

| | Guanidin | | Free Guanidin | | Creatin | | |
|---|---|---|---|---|---|---|---|
| | Total | as N. | Total | as N. | Total | as N. | Total Nitrogen |
| P. | ·391 | ·278 | ·0517 | ·0368 | ·303 | ·113 | 3·185 |
| N. | ·565 | ·402 | ·0839 | ·0597 | ·249 | ·0926 | 3·790 |

In proportion to the total nitrogen of the muscle the creatin nitrogen shows a rise of 45 p.c., while the free guanidin nitrogen shows a fall of 27 p.c. and the total guanidin nitrogen a fall of 18 p.c. This clearly

---

performed. It showed no symptoms until the 24th when there was a suggestion of water shake and slight depression. After that its condition appeared normal: it took food regularly and a slight fall in weight, probably due to confinement in the laboratory, was the only sign of any alteration in its condition. It was chloroformed on October 5th and considered along with the other normal animals. The probability is that parathyroid tissue was present in thymus (Morel (7)).

indicates that the changes in these constituents are quite independent of any alteration in the water and other non-nitrogenous constituents of the muscle.

The fact that the fall in the free guanidin nitrogen—0·0229—corresponds so closely with the rise in the creatin nitrogen—0·0204—seems to indicate a possible attempt to combine the liberated guanidin, but the fall in the total guanidin nitrogen is far in excess of the nitrogen in creatin and free guanidin and must indicate an escape of guanidin from the muscle, or a failure of the muscle to take guanidin from the blood.

The question of whether there is an increased formation of guanidin from other organs is being investigated.

## SUMMARY.

After parathyroidectomy there is a fall in the total and in the free guanidin of muscle, and a rise in the creatin, both absolutely and in relation to the total nitrogen.

The fall in the free guanidin nitrogen corresponds fairly closely with the rise in the creatin nitrogen and may indicate a synthesis of part of this nitrogen.

The fall in the total guanidin is far in excess of the nitrogen in free guanidin and in creatin and must indicate either a liberation of guanidin from the muscle or a failure in the power of the muscle to take up guanidin formed elsewhere, either of which may be correlated with the increase in the blood and the urine already demonstrated by Burns and Sharpe.

I have to thank Professor Noël Paton, at whose instigation the work was carried out, for help during its progress, and for performing certain of the operations. The others were performed for me by Mr A. Watson to whom my thanks are also due, as they are to Mr Burns and Mr Sharpe for help in the chemical analysis.

The expenses of the research were defrayed by the Medical Research Committee.

## REFERENCES.

(1) **Noël Paton, Findlay and Watson.** Quart. Journ. Exp. Physiol. 10. 243. 1917.
(2) **Burns and Sharpe.** Quart. Journ. Exp. Physiol. 10. 345. 1917.
(3) **Pekelharing.** Ztschr. f. physiol. Chem. 75. 207. 1911.
(4) **Burns.** Biochem. Journ. 10. 266. 1916.
(5) **Brown and Cathcart.** Biochem. J. 4. 420. 1909.
(6) **Koch.** J. Biol. Chem. 12. 303. 1912.
(7) **Morel.** Les parathyroïdes (Paris), 1912, p. 132.

A distinct decrease in the free guanidin is indicated, while the increase in the creatin noted in the previous experiments is confirmed.

Muscle contains other substances besides those containing nitrogen —*e.g.* water, fats, glycogen, etc., and the possibility of the variations in the percentage of guanidin being due to changes in those, had to be considered. The fact that the creatin is increased while the guanidin is decreased seems to indicate that the changes are not due to mere alterations in the proportion of the non-nitrogenous constituents. To settle this point the variations in the total nitrogen of the muscle were determined in a series of cats, normal and suffering from tetania parathyreopriva. The creatin content of the muscle was determined at the same time.

TABLE E.

| | Parathyroidectomised | | | Normal | |
|---|---|---|---|---|---|
| Cat | Nitrogen | Creatin | Cat | Nitrogen | Creatin |
| I. | 3·5 % | 0·322 % | II. | 4·10 % | 0·318 % |
| III. | 2·66 | ·382 | IV. | 3·50 | ·291 |
| V. | 3·78 | ·345 | VI. | 3·64 | ·244 |
| VIII. | 2·80 | ·360 | VII. | 3·92 | — |
| Average | 3·185 | ·352 | Average | 3·79 | ·284 |

This shows a fall of 16 p.c. in the total nitrogen. But since the fall in the total guanidin was found to be fully 30 p.c. and the fall in free guanidin 38 p.c.—there is a definite fall in relationship to the nitrogenous constituents of the muscle.

When the guanidin and creatin nitrogen throughout the whole series is calculated as percentage of the total, the following results are obtained:

TABLE F.

| | Guanidin | | Free Guanidin | | Creatin | | |
|---|---|---|---|---|---|---|---|
| | Total | as N. | Total | as N. | Total | as N. | Total Nitrogen |
| P. | ·391 | ·278 | ·0517 | ·0368 | ·303 | ·113 | 3·185 |
| N. | ·565 | ·402 | ·0839 | ·0597 | ·249 | ·0926 | 3·790 |

In proportion to the total nitrogen of the muscle the creatin nitrogen shows a rise of 45 p.c., while the free guanidin nitrogen shows a fall of 27 p.c. and the total guanidin nitrogen a fall of 18 p.c. This clearly

---

performed. It showed no symptoms until the 24th when there was a suggestion of water shake and slight depression. After that its condition appeared normal: it took food regularly and a slight fall in weight, probably due to confinement in the laboratory, was the only sign of any alteration in its condition. It was chloroformed on October 5th and considered along with the other normal animals. The probability is that parathyroid tissue was present in thymus (Morel (7)).

indicates that the changes in these constituents are quite independent of any alteration in the water and other non-nitrogenous constituents of the muscle.

The fact that the fall in the free guanidin nitrogen—0·0229—corresponds so closely with the rise in the creatin nitrogen—0·0204—seems to indicate a possible attempt to combine the liberated guanidin, but the fall in the total guanidin nitrogen is far in excess of the nitrogen in creatin and free guanidin and must indicate an escape of guanidin from the muscle, or a failure of the muscle to take guanidin from the blood.

The question of whether there is an increased formation of guanidin from other organs is being investigated.

## SUMMARY.

After parathyroidectomy there is a fall in the total and in the free guanidin of muscle, and a rise in the creatin, both absolutely and in relation to the total nitrogen.

The fall in the free guanidin nitrogen corresponds fairly closely with the rise in the creatin nitrogen and may indicate a synthesis of part of this nitrogen.

The fall in the total guanidin is far in excess of the nitrogen in free guanidin and in creatin and must indicate either a liberation of guanidin from the muscle or a failure in the power of the muscle to take up guanidin formed elsewhere, either of which may be correlated with the increase in the blood and the urine already demonstrated by **Burns** and **Sharpe**.

I have to thank Professor Noël Paton, at whose instigation the work was carried out, for help during its progress, and for performing certain of the operations. The others were performed for me by Mr A. Watson to whom my thanks are also due, as they are to Mr Burns and Mr Sharpe for help in the chemical analysis.

The expenses of the research were defrayed by the Medical Research Committee.

## REFERENCES.

(1) **Noël Paton, Findlay and Watson.** Quart. Journ. Exp. Physiol. 10. 243. 1917.
(2) **Burns and Sharpe.** Quart. Journ. Exp. Physiol. 10. 345. 1917.
(3) **Pekelharing.** Ztschr. f. physiol. Chem. 75. 207. 1911.
(4) **Burns.** Biochem. Journ. 10. 266. 1916.
(5) **Brown and Cathcart.** Biochem. J. 4. 420. 1909.
(6) **Koch.** J. Biol. Chem. 12. 303. 1912.
(7) **Morel.** Les parathyroïdes (Paris), 1912, p. 132.

# THE VELOCITY FACTOR IN CARDIAC WORK.

By C. LOVATT EVANS.

*(From the Institute of Physiology, University College, London.)*

It was shown in a recent paper by **Matsuoka** and the present writer (1) that in the computation of the work of the heart the velocity factor $\frac{mV^2}{2g}$ is often of considerable importance, and when large outputs are being dealt with forms a considerable percentage of the total work of the heart. In the calculations which were dealt with in that communication the velocity taken for these estimations was the mean velocity at the narrowest point close to the aortic orifice, in this case at the opening of the glass cannula inserted into the ascending aorta and having a diameter of 5 mm. at its narrowest point. It is this mean velocity which has hitherto been used in all calculations of cardiac work. Prof. **Starling** has suggested to me that it is not the mean blood velocity in the ascending aorta or at the aortic orifice which should be taken as a basis for calculation, but the velocity during the period of ventricular expulsion only. Since the velocity at the aortic orifice during the period of expulsion is also not uniform, but rises to a maximum and then declines again to zero, it would seem that for accurate calculation the velocity to be taken could be best arrived at by a mathematical treatment of cardiometer curves. But for present purposes such refinements as this would seem to be superfluous, since the velocity would be liable to an indeterminate amount of further variation owing to alteration of the sectional area of the orifice by stretching during maximal phases of pressure, as well as from other circumstances.

It will therefore be sufficient for present purposes to assume that the mean velocity in systole varies inversely as the duration of the period of expulsion. With the conditions which prevailed in the experiments already referred to, the period of systolic output would occupy not more than three-eighths of the cardiac cycle[1]. The mean velocity during expulsion will therefore be $2\frac{2}{3}$ times greater than the mean aortic velocity, and in accordance with this the expression $\frac{mV^2}{2g}$ in all the previous calculations where $V$ represents the mean aortic velocity should be multiplied by the square of 8/3 or by approximately 7 to arrive at the correct value. The work formula for both ventricles, when the rate of the heart does not greatly depart from the normal value which it has for the isolated organ, now becomes $W=7\left(\frac{QR}{6}+\frac{wV^2}{g}\right)$ instead of that previously given[1], $V$ representing the mean aortic velocity.

---

[1] Calculations from curves taken by **Patterson, Piper and Starling** (2) from hearts working under similar conditions indicate that it might be somewhat less than this, but the higher figure is taken in order to avoid over-estimating the effect now under consideration.

The effect of thus multiplying the velocity factor of both ventricles by 7 is to raise it to an order of magnitude comparable with that of the resistance ($QR$) expression; with low arterial pressures and high outputs in fact, it may happen with hearts under the conditions of our experiments that the greater part of the heart's energy is used in imparting velocity to the blood. When the output is small, say less than eight times the heart-weight per minute, the velocity factor is still small enough to be negligible. In Table I are given the values of the

TABLE I. Values of $\frac{7wV^2}{g}$ for dog's heart with aortic cannula of 5 mm. diameter.

| Output l. p. hr | Mean velocity m. p. sec. | $\frac{7wV^2}{g}$ kg.m. per hr (both ventricles) |
|---|---|---|
| 3 | 0·042 | 0·0038 |
| 12 | 0·169 | 0·253 |
| 36 | 0·51 | 6·72 |
| 48 | 0·67 | 15·4 |
| 60 | 0·845 | 30·7 |
| 72 | 1·02 | 53·7 |
| 84 | 1·18 | 84·2 |
| 96 | 1·35 | 125·0 |
| 108 | 1·52 | 179·0 |
| 120 | 1·69 | 245·0 |

---

[1] The formula previously given was $7\frac{QR}{6}+\frac{wV^2}{g}$. In cases where the relative duration of systole is much altered, the new formula given above must be modified to meet the altered conditions, and now becomes $W=7\frac{QR}{6}+\frac{w(VC)^2}{gE^2}$, where $C$=duration of cardiac cycle, and $E$=duration of period of expulsion. A further correction which might in some cases be of importance and lead to further modification of the formula, is introduced in cases where there is much difference between the sectional areas of the pulmonary and aortic orifices. The pulmonary orifice is usually held to be 3 or 4 mm. wider than the aortic, which is in the male human heart about 24 mm. in diameter *post mortem*; owing to the differences in pressure on the two sides of the heart, it is doubtful however whether this difference, or any difference at all, obtains during life, and this discrepancy is therefore not taken into further account here.

velocity expression for possible ranges of output of dog's hearts as used in the series of experiments with Matsuoka, and in which a cannula of known diameter was inserted into the aorta immediately beyond the valvular orifice. In Fig. 1 these results are shown graphically (*B*) and may be compared with the work done in overcoming the arterial resistance (*A*) at a pressure of 120 mm.; the total work done in kinetic and static forms together is also shown (*C*). It will be noted that at outputs of about 104 l. per hr. the values of the two modes of disposal of energy have become equal, while at still greater outputs the velocity factor rapidly outstrips the other.

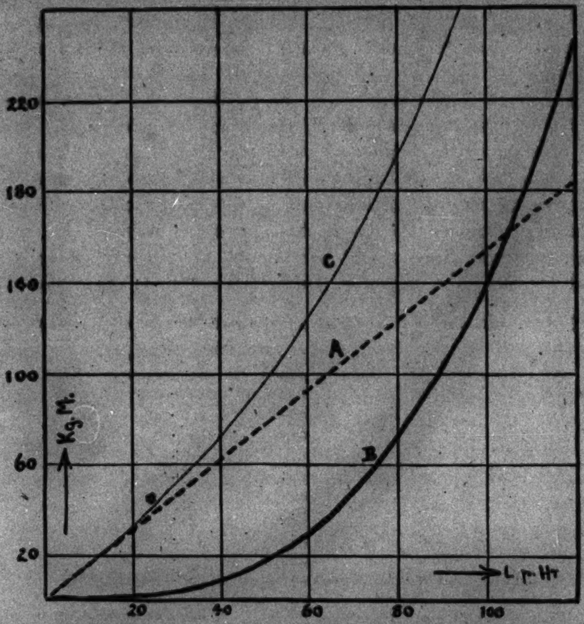


Fig. 1. Curves showing the relative importance of the kinetic and static factors when the arterial pressure is 120 mm. $A$ = values of $QR$. $B$ = values of $\frac{wV^2}{g}$ when $V$ represents velocity during systolic output. $C$ = total work done ($A+B$). The velocity factor, at first negligible, rapidly outstrips the resistance factor, as the output is raised.

*Effect of the foregoing considerations on previous results.* The effect of this enhanced importance of the velocity component on the results of previous experiments is to modify the amounts of work done by the hearts, and to raise the efficiencies of hearts working at high outputs.

TABLE II. Recalculations from the experiments bearing the same numbers given in *Journ. of Physiol.* 49. 402–403. 1915. The periods are here grouped together as shown in Column I and the average results are shown in the other columns.

| Periods | Arterial pressure | c.c. $O_2$ per hour | Output l. p. hr | Kg.m. work done p. hr | Kg.m. energy used p. hr | % efficiency |
|---|---|---|---|---|---|---|
| *Exp.* 7. | | | | | | |
| 1, 2, 3 | 80 mm. | 179 | 8·7 | 11·9 | 370 | 3·3 |
| 4, 5, 6 | „ | 185 | 16·3 | 20·7 | 383 | 5·5 |
| 7, 8 | „ | 212 | 27·8 | 37·1 | 437 | 8·5 |
| 9, 10 | „ | 287 | 52·0 | 81·6 | 593 | 13·8 |
| 11 | „ | 649 | 92·0 | 218·0 | 1343 | 16·3 |
| 12 | „ | 175 | 16·4 | 20·3 | 363 | 5·6 |
| *Exp.* 8. | | | | | | |
| 1, 2 | „ | 116 | 6·4 | 7·8 | 240 | 3·3 |
| 3, 4 | „ | 129 | 18·9 | 24·0 | 267 | 9·0 |
| 5 | „ | 247 | 53·4 | 87·0 | 509 | 17·1 |
| *Exp.* 9. | | | | | | |
| 1, 2 | „ | 97 | 14·6 | 18·2 | 201 | 9·0 |
| 3, 4, 5 | „ | 158 | 29·7 | 39·9 | 328 | 12·1 |
| 6, 7 | „ | 201 | 42·3 | 62·1 | 416 | 15·0 |
| 8, 9 | „ | 281 | 70·8 | 137·0 | 584 | 23·5 |
| 10, 11 | „ | 520 | 91·4 | 217·0 | 1078 | 20·4 |
| *Exp.* 10. | | | | | | |
| 1, 2 | „ | 140 | 17·0 | 21·2 | 291 | 7·3 |
| 3, 4 | „ | 159 | 33·8 | 46·7 | 329 | 14·2 |
| 5, 6 | „ | 299 | 81·0 | 175·0 | 620 | 28·2 |
| 7, 8 | 40 mm. | 98 | 17·0 | 11·0 | 202 | 5·5 |
| 9 | 80 „ | 119 | 16·8 | 20·7 | 247 | 8·4 |
| 10, 11 | 120 „ | 174 | 16·8 | 31·0 | 360 | 8·6 |
| 12 | 160 „ | 250 | 16·8 | 40·7 | 517 | 7·9 |
| 13 | „ | 334 | 37·7 | 99·0 | 693 | 14·3 |
| *Exp.* 11 | | | | | | |
| 1 | 100 mm. | 230 | 37·9 | 64·7 | 476 | 13·6 |
| 2 | 140 „ | 410 | 63·1 | 166·0 | 850 | 19·6 |
| 3 | 180 „ | 517 | 73·9 | 259·0 | 1070 | 24·2 |
| *Exp.* 12. | | | | | | |
| 1 | 100 mm. | 213 | 20·4 | 32·2 | 442 | 7·3 |
| 2 | 126 „ | 334 | 67·2 | 163·0 | 690 | 23·6 |
| 3 | 134 „ | 636 | 100·2 | 333·0 | 1310 | 25·5 |

The results of all those experiments in which the outputs were low are not altered. In the paper by **Matsuoka** and the present writer, the chief alterations are in Exps. 7 to 12, the results of which, corrected in the way already described, are given in an abbreviated form in Table II.

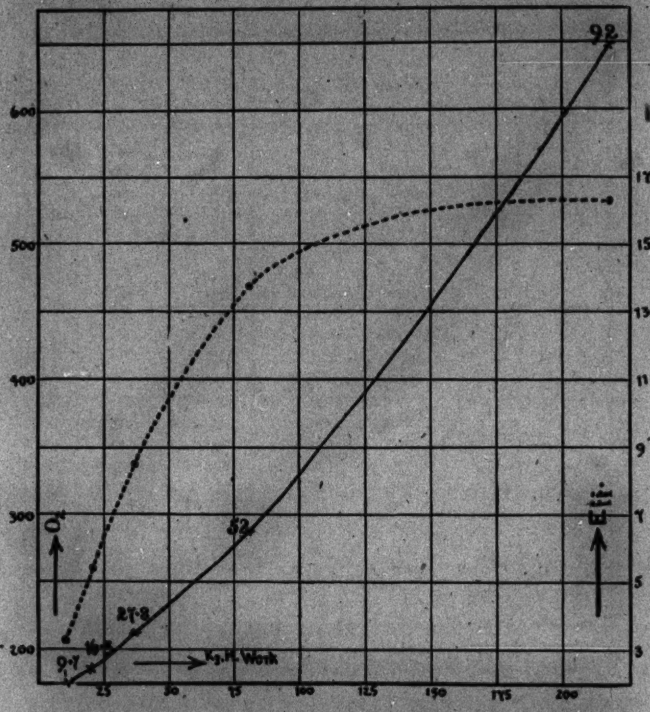


Fig. 2 (Exp. 7). Effect of varied output on oxygen usage (c.c. per hr—continuous line) and on % efficiency (dotted line). The figures on the oxygen curve indicate the hourly output in litres.

The results of Exp. 7 are shown graphically in Fig. 2 which should be compared with Fig. 3 of the former paper. It will be noted that the mechanical efficiency now calculated shows a continuous increase with increasing output, the rise being at first rapid and then more slow. The efficiency in some cases, however, still shows a falling-off when the output is raised beyond a certain limit. This is the case in Exp. 9, graphically represented in Fig. 3, and is presumably due to over-stretching of the fibres of the ventricular wall.

The main conclusions in the paper with **Matsuoka** on the effect of various conditions on the performance of the heart are not altered except as regards some of the quantitative results given. Thus the statement there made with regard to the maximum efficiency of the heart under the conditions of these experiments was that it was about 20 %; we now see that this figure has been often exceeded (Exps. 9, 10,

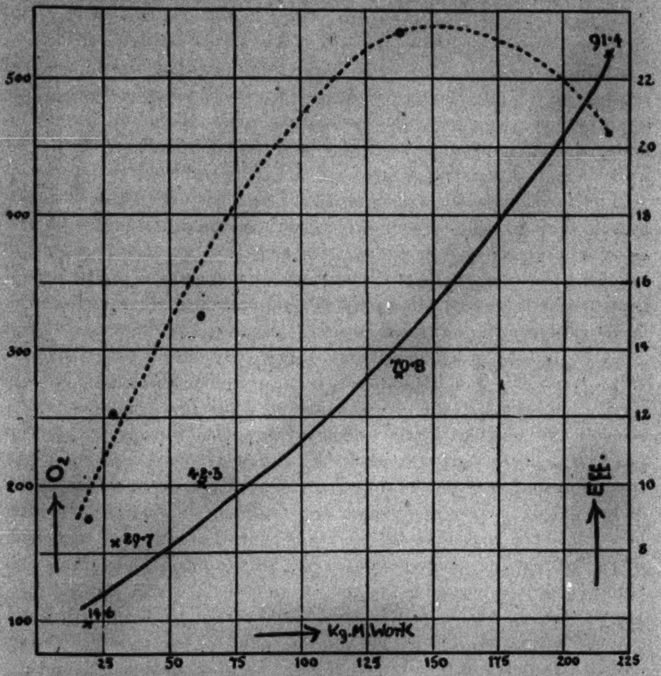


Fig. 3 (Exp. 9). Type of efficiency curve showing first a rise and then a fall of efficiency as the output increases. Curves and figures as in Fig. 2.

11 and 12) and the efficiency obtainable with optimum conditions probably lies in the neighbourhood of 30 %, *e.g.* 28·2 % in Exp. 10.

*Applications to the human heart.* In the absence of certain knowledge of the effective diameters of the aortic and pulmonary orifices in the human heart during life, it is hardly possible to make reliable calculations regarding the work done in giving velocity to the blood, since the work varies inversely as the square of the diameter of the outlet. It is moreover reasonable to suppose that in any individual heart these apertures, even when considered at the same phase of systole, are not constant in diameter, but vary according to the pressure within. With large outputs therefore, under conditions which prevail in the intact body, the accompanying rise of pressure would tend to relieve the work done in this respect.

Little is also known with regard to the velocity of the blood in the human aorta. If either the mean velocity at the aortic orifice, or the sectional area of the orifice itself were known, it would be possible to calculate the other from our knowledge of the volume output of the heart, which is known with a reasonable approach to accuracy from the investigations of Krogh(3) and others.

Nicolls(4) has calculated that the velocity of output past the aortic valves is ten times the mean velocity in the ascending aorta, and amounts to 320 cm./sec., but this calculation was made from premises which are no longer tenable. I have calculated that when the output is 60 c.c. per beat, and the aortic entrance has a diameter of 2·5 cms., and the time of output lasts for 0·3 sec., that the systolic velocity of output is about 40 cms. per second; these are data applicable to the normal heart when the body is at rest, and as is pointed out in every text-book of physiology, the kinetic energy given to the blood under these circumstances is a negligible fraction of the whole energy expenditure. Let us now consider a case where a large output is being dealt with, say the output of 21 litres per minute found in one case by Krogh and Lindhard(3); if the arterial pressure was low, say 100 mm. and the pulse rate and period of blood-expulsion were the same as obtained with the body at rest, the maximum velocity of blood would be about 196 cm./sec., and the velocity expression would amount to nearly 20 % of the total work of the heart. But these conditions do not obtain, for with such a large output the ventricle spends relatively longer in systole. Using our formula $W = \frac{7QR}{6} + \frac{w(VO)^2}{gE^2}$ for the calculation, we obtain the following result:

Assume the arterial pressure to be 130 mm., the duration of the cardiac cycle to be 0·5 sec., the period of expulsion to be 0·25 sec., then we have as the work of both ventricles, per min.

$$W = \frac{7 \times 21 \times 1{\cdot}7}{6} + \frac{21 \times ({\cdot}71 \times 0{\cdot}5)^2}{9{\cdot}8 \times (0{\cdot}25)^2} \text{ kg.m.}^{1}$$

$$= 41{\cdot}3 + 4{\cdot}3 \text{ kg.m. per minute.}$$

*i.e.* the expenditure of energy in kinetic form is still only about 9·5 % of the total. The advantage of the altered time-relations is evident.

Still clearer are the effects of a stenosis of the aortic opening. Let us suppose that the orifice be narrowed down to a diameter of 1 cm. In such a case the pulse rate is always slowed and a longer time is spent in systole, and these circumstances lead to a reduction of the blood velocity. Nevertheless, even under conditions of bodily rest, the velocity factor may easily represent half of the total work of the heart[2].

*The velocity factor in hearts under the influence of adrenalin.* It has been shown that the gaseous metabolism of the heart when under the influence of adrenalin is greatly increased(5), and Patterson has shown(6) that the duration of the isometric period is greatly shortened, being often reduced to half its normal value, while the time per minute spent in systole is also reduced. The great developments of endocardiac pressure in both ventricles is probably a result of this increase in the velocity of the blood expelled, and there seems little doubt that this factor plays a big part in determining the increase in the gaseous metabolism.

*Influence of heart rate on the efficiencies.* It must not be forgotten that in all these experiments we are dealing with the denervated heart, and that the heart as it beats in the intact animal would beat at rates

---

[1] The figure 1·7 represents the arterial pressure in terms of a column of blood: 0·71 is the mean velocity for the entire cycle, and is arrived at as follows:

$$\text{Sectional area of aortic orifice} = \pi \left(\frac{2{\cdot}5}{2}\right)^2 = 4{\cdot}9 \text{ sq. cm.}$$

21 l. per min. = 350 c.c. per sec.

$$\text{Velocity per second} = \frac{350}{4{\cdot}9 \times 100} = 0{\cdot}71 \text{ metre.}$$

The actual velocity of output is now about 140 cm. per second under the conditions assumed.

[2] Let us suppose that the output per beat in such a heart is 60 c.c. and the time of output is 0·5 second (this is certainly as long as it would ever be likely to be). The mean systolic velocity amounts to 152 cm./sec. and the distribution of the work done per heart beat is as follows:

$$QR = {\cdot}06 \times 1{\cdot}3 = {\cdot}078 \text{ kg.m.}$$

$$wV^2/2g = \frac{{\cdot}06 \times (1{\cdot}52)^2}{9{\cdot}8 \times 2} = {\cdot}071 \text{ kg.m.} = 48\,\% \text{ of total work.}$$

which, except in the case of the highest outputs, would be lower than those obtained with the use of the heart-lung preparation.

It is possible, by making certain assumptions, to allow provisionally for these differences, and to get an idea of what would be the approximate metabolism in cases where the rate resembled that in the normal animal. Thus, if we assume that (as is certainly the case for different rates due to slight differences of temperature), the gaseous exchanges are directly proportional to the rates, and if we further assume that the pulse rate steadily increases from the normal intact rate when the output is low, to the denervated rate when the maximum output is reached, then we shall find that the efficiencies at the lower outputs are considerably increased. Thus the following table shows such a recalculation in the case of Exp. 9. It is quite probable that the effect of this alteration in pulse rate is rather under- than over-estimated in this case.

TABLE III.

| | Unadjusted figures (Exp. 9) | | | Figures adjusted for rates | | |
|---|---|---|---|---|---|---|
| Output p. hr | Pulse rate p. m. | Oxygen usage c.c./hr | Efficiency % | Pulse rate p. m. | Oxygen usage c.c./hr | Efficiency % |
| 14·6 | 145 | 97 | 9·0 | 100 | 67 | 13 |
| 29·7 | 145 | 158 | 12·1 | 110 | 120 | 16 |
| 42·3 | 145 | 201 | 15·0 | 115 | 159 | 19 |
| 70·8 | 145 | 281 | 23·5 | 135 | 262 | 25 |
| 91·4 | 145 | 520 | 20·4 | 145 | 520 | 20·4 |

## SUMMARY.

It is shown that the energy expended in imparting velocity to the blood is very considerable, both under experimental conditions in animals and in the human heart during exercise.

## REFERENCES.

(1) **Evans and Matsuoka.** Journ. of Physiol. 49. 378. 1915.
(2) **Patterson, Piper and Starling.** Ibid. 48. 465. 1914.
(3) **Krogh and Lindhard.** Skand. Arch. f. Physiol. 27. 100. 1912.
(4) **Nicolls.** Journ. of Physiol. 20. 407. 1896.
(4) **Evans.** Ibid. 51. 91. 1917.
(6) **Patterson.** Proc. Roy. Soc. B. 88. 371. 1915.

# OBSERVATIONS ON THE ATROPHY OF DENERVATED MUSCLE. By J. N. LANGLEY, F.R.S. AND M. HASHIMOTO, M.D.

## CONTENTS.

PAGE
Errors due to differences in dissection . . . . . 16
Normal differences in weight of corresponding muscles . 18

BILATERAL DENERVATION.

1. Estimation of the original weight after bilateral denervation 24
2. Relative loss of weight of muscles on the two sides . . 26
3. Variations in weight in various pathological conditions . 31
*a.* Accidental injuries, p. 31. *b.* Protracted extension and protracted (moderate) flexion of ankle, p. 32. *c.* Section of tendons, p. 34.
4. Bilateral denervation experiments as criteria of the effect of treatment . . . . . . . . . . . . 37
5. Effect of some forms of treatment . . . . . . 41
*a.* Ionisation with calcium salt, p. 45. *b.* Ionisation with Ringer's fluid, p. 47. *c.* Ionisation with potassium salt, p. 48. *d.* Rhythmic flexion and extension. Massage, p. 50.

UNILATERAL DENERVATION.

1. Experimental errors in determining the effect of treatment 52
2. Relative rate of atrophy in different muscles . . . 54
3. The rate of atrophy . . . . . . . . . . 60
Notes on the condition of the muscle in atrophy . . 62

THERE are two methods which may be used in animals to determine to what extent a given treatment retards the atrophy of denervated muscle.

(1) The nerves in an animal may be cut on both sides of the body, treatment applied to the muscles on one side, and the weights of the corresponding muscles compared.

(2) The nerves to corresponding muscles may be cut on one side in different animals, treatment applied to the denervated muscles of

some of them, and the difference of weight of the corresponding denervated and non-denervated muscles compared in the treated and control animals.

*A priori* the former is the better method, for the rate of atrophy of corresponding muscles of the two sides of the body will probably differ less than that of corresponding muscles in different animals. Our main object then was to determine the character and extent of the errors involved in the bilateral method, and to make a few experiments on each of several ways of treatment with a view of seeing if any had a decided effect in delaying the atrophy. We have also made some observations on unilateral denervation. We took for investigation the muscles of the hind leg of the rabbit, since some observations have already been made on these animals by one of us(1) and a more direct comparison of results would be possible than if a different animal were taken. Nearly all the animals were cross bred tame rabbits of varying shades of brown or grey, and few had any marked sign of Lop-ear, Flemish, Himalayan, or other special breed. A few experiments were made with a pure strain of silver grey rabbits (cp. p. 47).

Most of the sources of error have been mentioned by Langley and Kato(2) and by Langley(1 and 3), but it will tend to clearness to mention them all in reference to the several points as they arise.

### *Errors due to differences in dissection.*

An error which applies to all comparisons of corresponding muscles is that the muscles may be cut with different amounts of tendon, fascia or other foreign tissue, and—when the muscle is attached almost directly to bone or to a tendon common to another muscle—with slightly different amounts of muscular tissue. The error, when corresponding muscles on the two sides in any one animal are taken, varies we think from 0 to about 30 mgms. in different muscles. In reckoning the percentage difference in the weight on the two sides, it is obvious that the less weight of the muscle, the greater will be the difference in percentage caused by a given small error. Thus an error in the method of dissection causing a difference of weight of 30 mgms. would be negligible in calculating the percentage difference of weight of a 10 gram muscle, but would cause a difference of 6 p.c. in the case of a muscle weighing only 0·5 gram. This must be borne in mind in considering the results on the several muscles. The error in percentage difference on the two sides, due to difference of dissection, is negligible in the gastrocnemius and plantaris and nearly so in the extensor digitorum communis.

It is slight in the tibialis anticus, if care be taken. In the other muscles there is more or less error. The popliteal, the flexor digitorum communis, and the peronei have to a varying extent to be scraped from bone, there is a varying amount of fat between the peronei, and the flexor digitorum communis is joined to the peroneus longus. In these the error we think is least in the flexor digitorum communis chiefly on account of its greater weight. Naturally, if sufficient care is taken, the corresponding muscles can be dissected out almost exactly alike, but if much time is spent on the dissection an error due to unequal evaporation will probably be introduced.

Clearly when the muscles of different animals are weighed at different times, there is a greater chance of the muscles being dissected in a somewhat different way than when the muscles are dissected at the same time. In order to be able to compare satisfactorily the results obtained in experiments made at different times, a constant method of dissection must be adopted. In some muscles, for example in the soleus and in the flexor digitorum communis, the tendons spread to such a large extent over the muscles that it is not possible to cut away accurately the tendon from the muscle. Thus some amount of tendon must be left and it is only a question of how much, all things considered, it is best to leave in order to obtain an equal amount at different times. We have proceeded as follows:

The upper tendons were cut close to their origin. The lower tendon of the soleus was cut where it joins the tendo Achillis. The other tendons were cut with the foot bent at right angles to the leg, one scissors blade being passed under them. The tendo Achillis (after separating the ligaments attached to it and the tendon of the soleus) was cut close to its insertion. The tendons of the flexor dig. com., the peronei and the extensor dig. I were cut at the point where they changed direction at the ankle. The tendons of the extensor dig. com. and of the tibialis anticus were cut where they run under the ligamentum cruris (annular ligament).

The four peronei do not necessarily atrophy at the same rate, but we have weighed them together, since if weighed separately the error in the percentage difference on the two sides is much increased.

By this method there is a not inconsiderable amount of tendon attached to all the muscles except the popliteal, it is relatively least in the tibialis anticus. In normal rabbits the weights of the tendons on the two sides differ very little, and if the percentage difference is calculated from the weights with the tendons cut off where the muscle ends, it is in most muscles practically the same as when the tendons taken as described above are included. But in the extensor dig. I, the extensor dig. com., and the peronei it may make a difference of ·5 to 1 p.c.

In Exp. 16 (Table II) after calculating the percentage difference in the usual way (see Table II) we cut off the tendons at their junction with the muscle and calculated their percentage difference less the weight of the tendons; the results were:

| | | Gastrocnemius | Plantaris | Flexor dig. com. | Extensor dig. I. | Extensor dig. com. | Peronei |
|---|---|---|---|---|---|---|---|
| Weight of tendon in grams | Rt. | ·216 | ·112 | ·195 | ·031 | ·058 | ·076 |
| | Lf. | ·205 | ·120 | ·207 | ·028 | ·048 | ·083 |
| p.c. difference of right from left: | | | | | | | |
| without tendons | | −5·7 | −5·0 | −5·7 | +1·5 | −3·0 | +5·4 |
| with tendons | | −5·4 | −5·1 | −5·7 | +2·1 | −2·5 | +4·7 |

The considerable expanse of tendon on the inner surface of the soleus can hardly be cut off in exactly the same way on the two sides. In this experiment, after weighing the muscle in the usual way, this piece of tendon was cut off: the weights were: Rt, ·168 grm., Lf., ·159 grm. The muscles after this removal differed in weight by 4 p.c., i.e. by 1·6 p.c. more than before the removal.

We adopted the method of dissection described above because at the time it seemed likely to give on the whole more comparable results than when the tendon was cut off at the junction with the muscle. In most of the muscles, the muscle fibres taper off to a fine point, and the degree of tapering varies in different animals, so that it seemed probable that by cutting the tendon at the junction, the error would be greater than by cutting at a fixed point. But in some pathological conditions there is considerable difference in the weights of the tendons on the two sides, and it is then necessary to cut them away at the junction with the muscle. When this was done it is mentioned.

## *Normal differences in weight in corresponding muscles of the two sides.*

Both the method of bilateral and unilateral denervation depend on the assumption that the muscles of the two sides of the body have practically the same weight. Langley and Kato(2) compared the weights of the combined tibialis anticus and extensor dig. com. in 12 rabbits in which the internal popliteal nerve had been cut on one side. In these cases there was probably some disuse atrophy on this side in the non-denervated muscles, so that there would be a tendency for the normal difference to be increased. They found a difference of less than 2·7 p.c. in eight cases, but in one case a difference of 12 p.c. Langley(1) has given three cases in which most of the muscles of the leg were weighed in normal rabbits: since these were on similar lines with our own, we shall incorporate the results with those we have obtained.

The rabbits referred to in this section were after death kindly given us by Mr Barcroft and Miss Dufton who had made some observations on them as to the effects of non-lethal doses of war gases. They were all bled before the muscles were weighed. In those given in Table I the muscles were in some cases taken immediately after death, and in others on the day following. The results were indistinguishable. In order to test the conclusion that allowing rigor mortis to occur did not appreciably affect the result, the muscles on the left side in Exp. 3 were taken immediately after death, and those of the right side 24 hours later, the body being kept in a cool cellar. It will be seen that the results are practically the same as in the other cases.

We give also two cases in which the muscles on the left side were cut out immediately after death, and those of the right, two days later. In one of these the difference in weight on the two sides was in general rather greater than in the other cases. In considering the average difference we leave both of these out of account.

In the observations given in Table II we weighed the animals before death; and after death (and bleeding) we weighed the stomach and intestines with their contents, in order to obtain additional facts as to the relation of the weight of the several muscles to the body weight. Further, in these and in most of the denervation experiments, after weighing the gastrocnemius and plantaris, the two muscles were isolated and weighed separately, since their weights might be expected to vary independently. As the upper end of the plantaris is attached to the gastrocnemius, what is taken from one in cutting the junction is added to the other: this possible error of dissection affects chiefly the plantaris on account of its less weight.

In the tables the body weights are given in kilograms and the weights of the muscles in grams to the nearest centigram. The muscles were weighed to a milligram and the percentage difference calculated from this; we give the percentage difference to one decimal place only. Obviously the percentage difference will vary according as the heavier or lighter muscle is taken as a standard, and the more the two muscles differ in weight the greater will be the divergence of the percentage in the two ways of reckoning. In the tables the actual weights of the muscles which are taken as a standard are given, those of the other side are given as percentage differences. When the muscles of one side

WEIGHT OF NORMAL MUSCLES ON THE TWO SIDES (RABBIT).

TABLE I.

| Exp. | Gastrocnemius + plantaris |  | Soleus |  | Flexor dig. com. |  | Popliteus |  | Extensor dig. I |  | Tibialis anticus |  | Extensor dig. com. |  | Peronei |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | left | p.c. diff. right | left | p.c. diff. right | left | p.c. diff. right | left | p.c. diff. right | left | p.c. diff. right | left | p.c. diff right | left | p.c. diff. right | left | p.c. diff. right |
| 1 | 6·08 | +0·7 | ·56 | −3·4 | 1·37 | +0·7 | ·51 | +0·6 | ·26 | +1·1 | 1·10 | +0·7 | 1·18 | +1·2 | ·94 | −3·5 |
| 2 | 8·67 | +2·0 | ·57 | +8·4 | 1·87 | +9·1 | ·77 | +1·7 | ·40 | −0·8 | 1·99 | −1·3 | 1·68 | +2·5 | 1·37 | +6·9 |
| 3* | 9·71 | +0·7 | ·95 | −2·0 | 2·11 | −0·3 | ·53 | +0·2 | — | — | 1·55 | +4·0 | 1·45 | +5·1 | 1·28 | +5·6 |
| 4 | 10·06 | −1·2 | ·85 | +1·8 | 2·49 | −5·5 | ·51 | −0·2 | — | — | 1·84 | +1·1 | 1·81 | −1·9 | 1·23 | +2·0 |
| 5 | 10·55 | +1·5 | ·64 | +9·0 | 2·62 | −2·5 | ·56 | +7·4 | ·36 | −1·1 | 1·75 | +2·6 | 1·75 | +3·5 | 1·41 | +3·2 |
| 6 | 11·35 | −0·7 | ·75 | −5·3 | 1·48 | +2·4 | ·53 | −4·7 | — | — | 1·21 | +4·3 | 1·22 | +1·2 | ·88 | +3·7 |
| 7 | 12·06 | −1·5 | 1·90 | 0 |  | 3·6 | −0·4 |  | ·57 | +6·2 | 2·33 | +1·8 | 2·45 | −4·2 | 1·95 | +1·0 |
| 8 | 12·12 | +0·6 | 1·10 | +5·0 | 2·90 | +0·2 | ·77 | −3·1 | — | — | 2·17 | +0·9 | 1·98 | +2·5 | 1·54 | +3·2 |
| 9 | 12·17 | −1·8 | ·98 | +0·9 |  | 3·9 | −6·9 |  | ·49 | −3·5 | 2·66 | −2·7 | 2·30 | +2·8 | 1·75 | −0·6 |
| 10† | 10·41 | +1·2 | ·99 | +2·2 | 3·16 | −1·0 | ·65 | −7·8 | — | — | 1·77 | +5·9 | 1·85 | +2·0 | 1·66 | −1·8 |
| 11† | 12·01 | +4·7 | 1·11 | +7·8 | 2·98 | +6·6 | ·82 | 0 | — | — | 2·14 | +7·5 | 2·24 | +6·5 | 1·86 | +1·8 |

* Left taken immediately after death; right one day later.

† Left ,, ,, ,, ,, right two days later.

TABLE II.

| | Gastrocnem. and plantaris | | Gastrocnem. | | Plantaris | | Soleus | | Flexor dig. com. | | Popliteus | | Ext. dig. I | | Tib. ant. | | Ext. dig. com. | | Peronei | | | Body weight less stomach intestine and contents |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp. | left. | p.c. right | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | lf. | p.c. rt | Body weight | |
| 12 | 6·40 | +3·5 | 4·44 | +2·2 | 1·95 | +6·9 | 0·76 | +2·2 | 1·78 | +5·2 | 0·45 | +0·4 | 0·22 | +5·9 | 1·35 | −7·0 | 1·34 | −1·9 | 1·03 | −1·5 | 1·37 | 0·94 |
| 13 | 7·64 | +1·2 | 5·24 | +0·8 | 2·39 | +1·5 | 0·88 | −1·3 | 2·36 | −0·7 | 0·64 | −1·9 | 0·38 | +1·3 | 1·59 | −3·4 | 1·69 | +1·7 | 1·41 | −4·5 | 1·56 | 1·13 |
| 14 | 9·12 | +1·4 | 6·40 | +1·9 | 2·72 | +0·2 | 0·95 | −1·6 | 2·43 | +0·9 | 0·63 | +0·9 | 0·44 | +5·5 | 1·83 | +2·2 | 1·74 | +4·1 | 1·47 | +4·3 | 1·79 | 1·21 |
| 15 | 9·25 | −3·3 | 6·51 | −2·5 | 2·74 | −5·3 | 1·09 | −2·8 | 2·39 | −5·2 | 0·71 | +1·3 | 0·42 | −3·8 | 1·81 | −4·3 | 1·81 | +2·1 | 1·63 | +0·4 | 1·87 | 1·32 |
| 16 | 10·54 | −5·3 | 7·28 | −5·4 | 3·26 | −5·1 | 0·98 | −2·4 | 2·94 | −5·7 | 0·77 | +3·0 | 0·48 | +2·1 | 2·23 | −3·2 | 2·19 | −2·5 | 1·69 | +4·7 | 1·93 | 1·48 |
| 17 | 11·16 | −1·0 | 7·69 | −1·4 | 3·47 | −0·3 | 0·89 | +1·2 | 3·13 | +2·5 | 0·70 | −7·0 | 0·33 | −5·2 | 2·09 | −3·2 | 1·92 | +0·2 | 1·36 | −2·1 | 2·13 | 1·59 |
| 18 | 12·78 | −1·0 | 8·67 | −1·0 | 4·11 | −1·2 | 1·22 | +0·4 | 3·75 | −2·8 | 0·74 | −1·8 | 0·52 | −1·0 | 2·45 | +2·4 | 2·51 | +0·6 | 1·86 | +2·2 | 2·32 | 1·87 |

were subjected to treatment, the muscles of the untreated side were taken as the standard.

In all the rabbits in Tables I and II, and in the three the weight of the muscles of which have been previously published(1), some of the muscles on one side were heavier and some were lighter than on the other side. Consequently if after denervation on both sides, the muscles on one side are all heavier than on the other, there is a fair probability that the atrophy has been unequal. The number of cases in which the flexor muscles were heavier on the left side was practically the same as that in which they were heavier on the right side. The extensor muscles were more often heavier on the right than on the left, but possibly the ratio would be altered by further observations.

The total number of muscles, or small groups of muscles, weighed was 153, including sixteen already published(1) and eight from Table XI. The degree of frequency of given ± percentage differences between the several muscles of the right and left side is shown in Table III.

The data given in Table III give a more or less satisfactory basis for estimating the correction to be made in the weight of muscles after treatment so far as concerns the original difference of weight in the muscles of the two sides. Taking the results together, it will be seen that in 75 p.c. of the cases the difference of weight of the muscles on the right from those on the left side was less than 4 p.c.; and that it was more than 5·9 p.c. in only 8 p.c. of the cases. Results of the same order, though from less numerous weighings, were obtained by one of us in the cat(3). The difference may be spoken of as the difference between the weight of the muscles on the two sides, since it is practically the same on the left if the right is taken as standard.

Whilst it is clear that in normal animals the majority of the muscles of the leg differ but little in weight on the two sides, it is also clear that any one muscle in any one animal may be about 6 p.c. to about 9 p.c. heavier on one side than on the other, the error due to difference in dissection being included, so that if the treatment is confined to single muscles there is great uncertainty in interpreting the results. So far as equality of weight on the two sides is concerned the gastrocnemius appears to be the most favourable muscle for experiment; unfortunately this muscle as a whole is less accessible than several of the others. The limits of percentage difference found in our experiments are given in the last column of Table III, these of course might be extended by further observations.

The mean percentage differences in the weight of the muscles on the

TABLE III. Percentage difference of weight of the muscles of the right side from those of the left in undenervated rabbits.

| Number of cases | Muscles | 0—1·9 p.c. | 2—3·9 p.c. | 4—5·9 p.c. | 6—7·9 p.c. | 8—9·1 p.c. | Mean difference ± from 0 | Limits of difference |
|---|---|---|---|---|---|---|---|---|
| 16 | Gastrocnemius + plantaris | 13 | 3 | 1 | 0 | 0 | 1·7 | −5·2 to +3·5 |
| 4 | Gastrocnemius + soleus ... | 1 | 2 | 1 | 0 | 0 | — | −2·1 to +3·6 |
| 8 | Gastrocnemius ... ... | 5 | 2 | 1 | 0 | 0 | 1·9 | −5·4 to +2·2 |
| 10 | Plantaris ... ... ... | 6 | 1 | 2 | 1 | 0 | 3·0 | −5·3 to +6·9 |
| 16 | Soleus ... ... ... | 8 | 5 | 2 | 0 | 2 | 3·0 | −5·3 to +8·4 |
| 16 | Flexor dig. com. ... ... | 6 | 6 | 4 | 0 | 1 | 2·8 | −5·7 to +9·1 |
| 2 | Flexor dig. com. + popliteus | 1 | 0 | 0 | 1 | 0 | — | — |
| 14 | Popliteus ... ... ... | 9 | 3 | 1 | 2 | 0 | 2·4 | −4·7 to +7·4 |
| 12 | Extensor dig. I ... ... | 5 | 3 | 3 | 1 | 0 | 3·1 | −3·5 to +6·2 |
| 19 | Tibialis anticus ... ... | 5 | 9 | 4 | 2 | 0 | 3·2 | −7·0 to +6·2 |
| 19 | Extensor dig. com. ... | 9 | 8 | 3 | 0 | 0 | 1·8 | −4·2 to +5·1 |
| 18 | Peronei ... ... ... | 4 | 8 | 5 | 2 | 0 | 3·3 | −4·7 to +6·9 |

In the following totals of muscles or small groups of muscles weighed, the gastrocnemius + plantaris when they were also weighed separately (8 cases) are omitted.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 153 | | 67 | 48 | 26 | 9 | 3 | — | — |
| | Percentage to nearest whole number ... ... ... | 44 | 31 | 17 | 6 | 2 | — | — |

two sides are given in the last column but one in Table III. These can only be used as a basis of comparison if a large number of experiments on the effect of treatment are made.

When pathological conditions are recognisable, the observations must of course be discarded. It is to be remembered moreover that minor injuries in early life may leave no recognisable sign, and yet have been sufficient to modify the growth of the muscle.

## BILATERAL DENERVATION.

### 1. *Estimation of the original weight of muscles after bilateral denervation.*

The actual degree of atrophy in the denervated muscles is of no moment as regards the effect of treatment, when the muscles of both sides are denervated, and those of one side only are treated, provided the treatment is of sufficient duration to make certain that considerable atrophy has taken place. Nevertheless it is of some interest to be able to form a general idea of the degree of atrophy. For any single case this can only be done within wide limits of possible error. There are two methods of estimating the original weight and so the degree of atrophy.

(*a*) An estimate of the initial weight of the several muscles may be made from the data of the weight of the muscles which goes with a given body weight in normal animals.

Whilst in general we should expect that the muscle weight would increase with the body weight, we should also expect the ratio of these to show considerable fluctuation, partly in consequence of the body weights not being comparable—since the states of nutrition and amount of food in the alimentary canal will differ—and partly in consequence of a difference in breed. Accurate estimations of the body weight were only made in the observations given in Table II. In these, an attempt was also made to determine how far the ratio was affected by the different amounts of food in animals taken at random. After death the stomach or intestine with their contents were weighed, and then deducted from the total body weight. In questions of this nature seven observations can only give a very approximate determination of the general relation of muscle weight to body weight, or of the range of possible variations. We may however consider first the observations given in Table II and then consider the modifications of the results which our other observations suggest. In Fig. 1 we give the curve showing the relation of the weight of the gastrocnemius + plantaris to the body weight, and to the body weight less the stomach, intestines, and their contents. It will be seen that the ratio is but little more constant in the latter than in the former and we conclude that the error due to variations in the amount of food is largely over-ridden by other factors. It will also be seen that the muscle weight increases in approximately direct ratio with the body weight. This we think is the chance of the rabbits taken, and that if the observations had been more numerous much wider variations in the ratio would have been found. This opinion is based

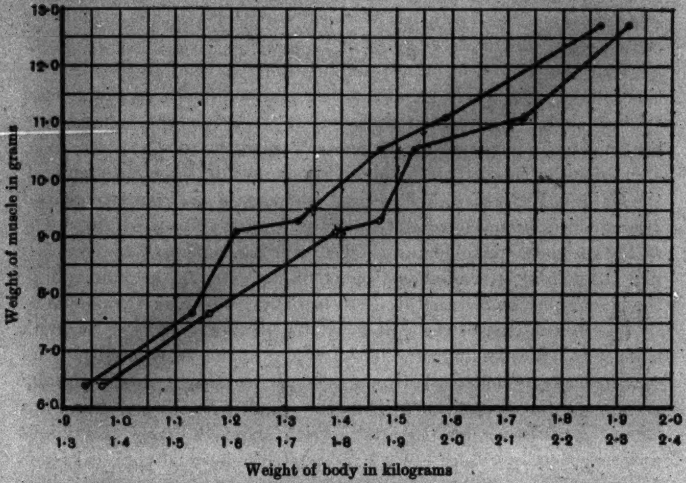


Fig. 1. Relation of weight of gastrocnemius + plantaris to body weight from the data given in Table II. In the abscissa, the upper line of figures give the body weight less that of the stomach, intestines and their contents, and those in the lower line give the gross body weight.

partly on the observations made after unilateral denervation, and partly on those given in Table I. In the observations given in Table I, we could only determine the body weight approximately, as the animals were given us after removal of the heart and lungs, and loss of more or less blood. But the body weight as estimated indicated that the weight of the gastrocnemius + plantaris accompanying a given body weight was somewhat greater than in the observations given in Table II, and that in two cases (Exps. 6 and 9) it was much greater, especially in one of these (Exp. 6). It will be noticed that in Exp. 6 the gastrocnemius + plantaris and the soleus are much heavier compared with the remaining muscles than in the other cases. Similarly we think that the unilateral denervation cases (Table XIV) indicate an average curve somewhat above that of Fig. 1 and wide variation in individual instances.

With regard to the other muscles, there is a fairly constant ratio between muscle weight and body weight in the case of the tibialis anticus, of the extensor digitorum communis, and a somewhat more variable one in the case of the flexor digitorum communis. In the remaining muscles the ratio has wider variation, but the chief irregularity is due to one experiment (Exp. 17).

Thus the relation of muscle weight to body weight in the observations recorded in Table II, whilst they give some idea of the general relation, do not allow more than a very rough estimate to be formed of the weight of a given muscle in any one experiment.

It may be noted that the observations tend to show that between the limits of 1 to 3 kilos. body weight, an increase of 1 kilo. is in general accompanied by an increase of rather more than 100 p.c. in the weight of the gastrocnemius + plantaris, the flexor dig. com., and the extensor dig. I; by an increase of about 100 p.c. in the extensor dig. com. and in the peronei; by an increase of rather less than 100 p.c. in the popliteus.

(*b*) When some of the muscles of the leg are denervated on both sides, a rough estimate of the initial weight of the denervated muscles may be made by noting what weights of the denervated muscles usually accompany the ascertained weights of the undenervated muscles. Thus when the gastrocnemius + plantaris weigh about 12 grams, the tibialis anticus generally weighs 2 to 2·5 grams. If the internal popliteal nerve has been cut on both sides and the undenervated tibialis anticus is 2 to 2·5 grams, the original weight of the gastrocnemius may be taken as about 12 grams. The estimate would probably be too low, since as we shall see there is evidence in such circumstances of some disuse atrophy in the tibialis anticus. Obviously by this method also there is a wide range of possible error in any individual case.

### 2. *Relative loss of weight of the muscles on the two sides.*

In all denervation experiments the possible effects of the other pathological conditions which are apt to occur have to be borne in mind. After section of sensory nerves, the tissues are less resistant to injurious influences than in the normal state. Both the absence of sensation and the partial paralysis lead to abnormal pressure and to abnormal friction on certain parts of the skin. Even when both popliteal nerves are cut the animal can still jump to a certain extent by the use of the thigh muscles, but it alights heavily on the heel, and a sore develops on it in practically all cases unless precautions are taken. At first we tried to prevent this by bandaging, the bandage however tended to compress the skin veins and cause œdema. Later we fixed thick plaster on the heel, this generally prevented the formation of a sore. The inflammation on the heel may spread upwards, it will then cause connective tissue growth passing along the Achilles tendon and may affect the muscles attached to it; if the sore is deep it will similarly affect the flexor dig. com. It causes also in such cases congestion of the vessels, chiefly in the posterior surface of the leg. Deep bedding reduces the chance of a heel sore, but it has the drawback that, in movement, the toes are apt to catch in it, put strain on the extensor muscles and cause sores, though comparatively slight, on the dorsal surface of the toes. Inflammation on the dorsal surface of the toes may spread up the tendon of the extensor dig. com. In two heavy rabbits, there was dry gangrene of the toes, apparently this was due to the toes being doubled up for some length of time in the sitting posture—the usual posture in the denervated as in the undenervated leg—and thus the blood supply cut off.

As we shall see, more or less considerable percentage difference in the weights of some of the corresponding muscles occurs when there is distinct connective tissue growth, or congestion of the muscles of the leg. But it is difficult to determine whether pathological changes in the foot which do not visibly spread to the leg have any effect on the muscle weight. If they have any effect, we think it can only be slight.

The sciatic nerve can be cut in three to five minutes. A cut 2 to 2½ cm. long is made in the skin of the posterior surface of the leg about 1 cm. from the posterior edge and midway in the thigh. With the aid of fine scissors and forceps the tissue lying along the median edge of the conspicuous vein is cut close to it (it is sometimes better to cut the superficial connective tissue of the vein laterally and to pull it to the median side). Forceps are passed under the biceps femoris to free it from the underlying tissue. On retracting the muscle, the sciatic is seen. Curved forceps are passed under it, and the nerve cut between the blades. For the popliteal nerves the skin cut is a little lower. The skin wound is dry in 1 to 2 days.

In two of our eight experiments on bilateral denervation without treatment, there were marked pathological changes in the leg, and these will be dealt with in the following section. There was a varying degree of pathological change in one or other or both feet in five out of the remaining six cases, but in two only was there any visible change in the leg. In Exp. 3 there was a large sore on the right heel, and a little connective tissue growth around the lower end of the Achilles tendon. In Exp. 6 there was temporary inflammation of the skin on both sides; chiefly on the right.

The popliteal nerves were cut on both sides in Exps. 1, 2, and 3, in the upper part of the popliteal space.

*Exp.* 1. Large sore on right heel.

*Exp.* 2. Dry sore on right heel.

*Exp.* 3. See text.

*Exp.* 4. Internal popliteal nerves cut on both sides, and also the right external popliteal. Some inflammation on dorsal surface of toes but skin intact.

*Exp.* 5. Both external popliteal nerves cut.

*Exp.* 6. Section at the knee of the external portion of the peroneal nerves supplying the tibialis ant. and extensor dig. com.; compression of the nerves to the peronei, slight on the right, stronger on the left side. The skin wound was well joined and dry on the second day but on the following day the sutures were torn out in movement, the wound became slightly infected, chiefly on the right side, and took 10 days to heal. In order to minimise the effect of this, the animal was not killed till 30 days after denervation. The right peronei were 16 p.c. heavier than those on the left, the degree of nerve degeneration corresponded with the weights. It may be noted that the extensor dig. com., judging by its weight, atrophied very little. For some other anomalies in this muscle cp. p. 56.

In nearly all our experiments, the denervated muscles were exposed in the anæsthetised animal just before death in order to note the extent of the fibrillation; in some cases their excitability was tested, or the effect of ionisation on the fibrillation. In some cases the exposure and stimulation were unequal on the two sides, but we have not been able to trace any connexion between this and the relative muscle weight.

The excess of the percentage differences in these experiments over those in normal animals represents the difference in the rate of atrophy which occurred on the two sides. The excess might be due either to the conditions being different on the two sides, or to an intrinsic difference in the rate of atrophy of corresponding muscles. Some part of the excess was we think undoubtedly due to slight unilateral inflammation. It is noticeable that all three cases in which the percentage difference was over 8, were in the two experiments in which there was some recognisable inflammation in one leg. Further in the cases to be dealt with later in which there was treatment on one side, but no trophic change, the percentage differences were on the whole smaller than those in Table IV. We infer that the difference in the intrinsic rate of atrophy of corresponding muscles on the two sides is small.

Although the experiments are not numerous enough to allow satisfactory percentages to be calculated of the different degrees of difference, they afford some basis for comparison. In drawing up the Table there is a primary difficulty. In the seven normal rabbits (Table II) in which

TABLE IV. Relative loss of weight after denervation on both sides.

The weights of the muscles which were denervated are in black type.

| Exp. ... ... ... | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Duration of denervation | 21 days | | 22 days | | 24 days | | 23 days | | 21 days | | 30 days | |
| Body weight at operation | 2·24 | | 1·46 | | 1·97 | | 2·32 | | 1·98 | | 1·78 | |
| ,, ,, ,, death ... | 2·32 | | 1·70 | | 1·78 | | 2·37 | | 2·10 | | 2·12 | |
| ,, ,, less gut ... | 1·71 | | — | | — | | 1·90 | | 1·53 | | 1·51 | |
| | lf. | p.c. diff. rt | lf. | p.c. diff. rt | lf. | p.c. diff. rt | lf. | p.c. diff. rt | lf. | p.c. diff. rt | lf. | p.c. diff. rt |
| Gastrocnemius + plantaris | **7·25** | +1·7 | **3·98** | +1·1 | **4·16** | +2·9 | **6·54** | + 1·0 | **9·02** | +1·5 | **10·76** | +2·9 |
| Gastrocnemius ... ... | **4·93** | +4·6 | — | — | — | — | **4·36** | + 4·6 | — | — | — | — |
| Plantaris ... ... ... | **2·31** | −4·2 | — | — | — | — | **2·15** | − 6·3 | — | — | — | — |
| Soleus ... ... ... | **1·19** | +1·5 | **0·60** | −5·7 | **0·50** | −9·6 | **0·86** | + 5·6 | **1·04** | +6·9 | **1·13** | +6·7 |
| Flexor dig. com. ... ... | **2·42** | −7·6 | **1·31** | −2·3 | **1·42** | +1·5 | **2·55** | − 1·9 | **2·50** | +0·5 | **3·41** | −2·1 |
| Popliteus ... ... ... | **0·62** | −3·7 | **0·37** | −3·5 | **0·34** | −8·7 | **0·74** | 0 | **0·80** | +1·1 | **0·74** | +7·8 |
| Tibialis anticus ... ... | 2·08 | −4·8 | 1·33 | −0·7 | 1·66 | −0·4 | 2·37 | −36·5 | **1·14** | +4·6 | **1·65** | −5·8 |
| Extensor dig. com. ... | 2·05 | +1·0 | 1·53 | −2·5 | 1·70 | +0·9 | 2·39 | −30·2 | **1·22** | −0·7 | **2·32** | −8·8 |
| Peronei ... ... ... | 1·83 | +7·2 | 1·23 | −0·2 | 1·52 | −7·2 | 1·90 | −36·4 | **1·07** | +6·1 | — | — |

the gastrocnemius and plantaris, after being weighed together, were weighed separately, the percentage difference of the two muscles, though they differed somewhat, were of the same sign. In the two experiments after bilateral denervation, in which a similar proceeding was adopted, the percentage differences of the two muscles were of opposite sign, so that the smaller percentage difference found in the cases in which the two muscles were only weighed together may be illusory. We have however drawn up Table V in the same way as Table III, omitting the combined weights when the separate weights were taken.

TABLE V. Percentage difference of weight of the muscles of the right side from those of the left after bilateral denervation in 21 to 30 days (rabbits).

| Number of cases | | 0–1·9 | 2–3·9 | 4–5·9 | 6–7·9 | 8–9·1 |
|---|---|---|---|---|---|---|
| 2 | Gastrocnemius + plantaris... | 1 | 1 | — | — | — |
| 2 | Gastrocnemius ... ... | — | — | 2 | — | — |
| 2 | Plantaris ... ... ... | — | — | 1 | 1 | — |
| 4 | Soleus ... ... ... | 1 | — | 2 | — | 1 |
| 4 | Flexor dig. com. ... ... | 2 | 1 | — | 1 | — |
| 4 | Popliteus ... ... ... | 1 | 2 | — | — | 1 |
| 2 | Tibialis anticus ... ... | — | — | 2 | — | — |
| 2 | Extensor dig. com. ... | 1 | — | — | — | 1 |
| 1 | Peronei ... ... ... | — | — | — | 1 | — |
| 23 | | 6 | 4 | 7 | 3 | 3 |
| | Percentage difference to nearest whole number | 26 | 17 | 30 | 13 | 13 |
| | Percentage difference if 1 p.c. added to each of the 153 normal cases of Table III | 24 | 39 | 18 | 15 | 4 |

It will be seen that 43 p.c. of the corresponding muscles, or small groups of muscles, had a less percentage difference than 4 p.c.; 71 p.c. had a less difference than 6 p.c., and 26 p.c. had a percentage difference of 6 to 9·1 p.c. A slight difference in the rate of atrophy on the two sides makes a considerable modification in the percentage differences due to differences in initial weight. The extent of this may be indicated by adding 1 p.c. to each of the 153 cases of the percentage differences in normal rabbits (Table III). The results are given in the last line of Table V. In view of this and of the practical certainty that the accessory pathological conditions which occurred in some of the experiments on bilateral denervation caused some variation in the atrophy, it may be concluded that if such conditions can be prevented from occurring, the variations from the percentage differences in normal animals will, with a possible error of 1–1·5 p.c., represent as a rule the effect of treatment.

### 3. *Variations in weight in some pathological conditions.*

#### (*a*) *Accidental injuries.*

Four of the experiments on bilateral denervation had to be discarded on account of accessory pathological states. These are of importance as showing the variations which may occur in such conditions. No two were the same. In one case there was early fracture of the right metatarsus causing inflammation and œdema of the leg and the animal was killed as soon as the fracture was found. In another, there was slow development of dry gangrene of the toes, and the animal was killed after 10 days; there was connective tissue growth about the Achilles tendon and congestion of the vessels of the leg, chiefly on the right side. Both were heavy rabbits—apparently with a strong strain of Flemish breed—and the accidents were we think due to their weight. These may be taken as showing the effect of brief acute inflammation in an early stage of denervation, and of less acute inflammation of rather longer standing. The other cases illustrated the necessity of post-mortem examination of the bones. In one of these there was enlargement of the lower end of the right tibia, in the other there was an old and healed fracture of the right tibia.

*Exp.* 1. Large rabbit. Right metatarsus broken on the night of the 3rd day causing acute inflammation and œdema of the leg. The animal was killed on the following morning. On the right side intermuscular connective tissue in general gelatinous, but not over flexor dig. com.

*Exp.* 2. Large rabbit. Dry gangrene of the toes slowly developed, affecting three toes on the right side, and one slightly on the left, probably in consequence of the animal sitting with the insensitive toes doubled up. There was some increased connective tissue growth about the Achilles tendon on both sides, but considerably greater on the right.

*Exp.* 3. Post-mortem, a swelling of long standing was found on the lower end of the right tibia, and the tibia was somewhat curved.

*Exp.* 4. Post-mortem, a rather large swelling was found in the middle of the left tibia caused by an old fracture which had healed.

The results in these experiments present, as a whole, a striking contrast to those in Table IV. In both cases of inflammation, all the muscles on the affected side were heavier than those on the opposite side, and in that of acute inflammation most of them were considerably heavier. This may have been due to the absorption of water, there was however no œdema in Exp. 2. In the experiments in which there was long standing injury to the tibia there was more or less variation from

TABLE VI. Accidental pathological conditions in addition to denervation. The weights of the denervated muscles are in black type.

| | Acute inflammation right | | Sub-acute inflammation right | | Deformation of right tibia | | Old fracture of left tibia | |
|---|---|---|---|---|---|---|---|---|
| Exp. ... ... ... | 1 | | 2 | | 3 | | 4 | |
| Duration of denervation | 4 days | | 10 days | | 21 days | | 23 days | |
| Body weight at operation | 3·55 | | 3·5 | | 1·78 | | 2·12 | |
| " " death ... | 3·52 | | 3·5 | | 2·12 | | 2·42 | |
| " " less gut ... | — | | — | | 1·51 | | 1·85 | |
| | lf. | p.c. diff. rt | lf. | p.c. diff. rt | lf. | p.c. diff. rt | rt | p.c. diff. lf. |
| Gastrocnemius + plantaris ... | **16·41** | +12·3 | **15·56** | + 4·6 | **5·06** | + 2·9 | 12·30 | − 7·5 |
| Gastrocnemius ... | — | — | — | — | **3·46** | + 5·3 | 8·75 | − 7·8 |
| Plantaris ... ... | — | — | — | — | **1·59** | − 2·3 | 3·55 | − 6·9 |
| Soleus ... ... | **1·71** | +27·9 | **1·26** | +11·2 | **0·73** | − 2·3 | 1·36 | − 8·5 |
| Flexor dig. com. } | **5·14** | + 3·9 | **3·57** | + 7·7 | **1·68** | + 1·9 | 3·32 | − 3·8 |
| Popliteus ... } | | | **1·11** | + 6·5 | **0·46** | − 5·0 | 0·94 | +13·3 |
| Tibialis anticus ... | **3·21** | +28·4 | **3·36** | +11·5 | **1·28** | − 8·8 | 1·29 | +13·1 |
| Extensor dig. com. | **3·36** | +27·1 | **3·48** | + 4·5 | **1·67** | −11·9 | 1·78 | − 6·4 |
| Peronei ... ... | **2·01** | +12·2 | **2·09** | + 3·7 | **1·19** | − 8·1 | 1·38 | − 5·3 |

the results of uncomplicated bilateral denervation. In Exp. 3 the extensors only were affected; in Exp. 4 the variation affects nearly every muscle.

### (b) *Protracted extension and protracted (moderate) flexion of ankle.*

The effects usually ascribed to protracted extension of a joint are connective tissue growth at the joint and in time permanent elongation of the stretched muscles. We made three experiments to observe the effect on the relative weight of the leg muscles of protracted extension of one ankle for varying periods after bilateral denervation. Although a rabbit occasionally lies with the ankle more or less extended, and sometimes sits with the leg at right angles to the foot, the normal position of the ankle is one of flexion, and long continued extension is very abnormal. In each case there was considerable connective tissue growth around the joint. In Exp. 1 there was rupture of muscle fibres in the upper part of the extensor dig. com. probably in consequence of the toes being left uncovered by the splint. There was also recurring œdema of the leg and foot owing to tightening of the bandages in movement; in consequence the animal was killed after 11 days. In Exp. 2 there was some adhesion of the sheath of the tibialis anticus to the muscle. In Exp. 3 the adhesion of the sheath of the tibialis anticus was greater and there was also adhesion between the tibialis anticus and extensor digitorum; both muscles were about 5 p.c. heavier than on the unextended side, but the exact weights could not be obtained on account of the intermixture of connective tissue and muscle on the surface.

We made also one experiment in which the foot was kept by a bandage from being extended at any time; the degree of flexion was somewhat less than that occurring in the ordinary sitting posture.

*Exp.* 1. Sciatics cut in middle of thigh; internal saphena also cut. Splint on left ankle, not covering toes; the movements of the animal caused at times tightening of the fastening of the splint at the back of the leg, setting up œdema. The splint was kept on in all for about 5¼ days; and the animal killed after 11 days. Congestion throughout the leg.

*Exp.* 2. Sciatics cut at great trochanter. Splint on left ankle extending over the toes. The splint was kept on for 6–7 hours a day after the first few days. At death the left foot could not be flexed as far as the right. There was connective tissue growth about the lower tendons of the tibialis ant. and extensor dig. com. and some adhesion of the sheath of the tibialis anticus to the outer surface of the muscle. The other muscles and tendons did not differ to the eye from the muscles of the other side. No marked congestion in the posterior part of the leg.

*Exp.* 3. Operation and method as in Exp. 2. The connective tissue about the ankle joint was much greater, there was considerable adhesion of the sheath of the tibialis anticus to the muscle, and adhesion between the tibialis ant. and the extensor dig. com. The other muscles and tendons apparently the same on the two sides.

*Exp.* 4. Both sciatics cut. A strip of plaster was fastened to the under surface of the right foot and to the knee, so that in the movements of the animal the tibialis ant. and extensor dig. com. could not at any time be stretched. At death, the right ankle joint did not move quite as freely as the left.

TABLE VII. Protracted extension, and protracted moderate flexion of ankle. Bilateral section of sciatic. Extension in Exps. 1, 2, 3 on the left side. Flexion in Exp. 4 on the right side.

| Exp. ... ... ... | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Duration of denervation | 11 days | | 20 days | | 21 days | | 20 days | |
| Body weight at operation | 1·89 | | 2·15 | | 2·05 | | 1·87 | |
| „ „ death ... | 1·74 | | 2·14 | | 2·03 | | 2·97 | |
| „ „ less gut ... | — | | 1·52 | | 1·46 | | 1·56 | |
| | rt | p.c. diff. lf. | rt | p.c. diff. lf. | rt | p.c. diff. lf. | lf. | p.c. diff. rt |
| Gastrocnemius + plantaris | — | — | 6·23 | – 7·3 | 6·16 | – 0·5 | 5·38 | +0·3 |
| Gastrocnemius ... ... | 4·71 | +10 | 4·21 | – 6·1 | 4·02 | – 0·4 | 3·64 | –2·0 |
| Plantaris ... ... ... | 2·04 | +13 | 2·00 | – 9·7 | 2·14 | – 0·6 | 1·71 | +5·2 |
| Soleus ... ... ... | 0·83 | + 5·4 | 0·74 | + 4·3 | 0·64 | + 5·5 | 0·72 | +0·4 |
| Flexor com. ... ... | 1·92 | – 5·9 | 2·02 | – 6·3 | 2·07 | + 1·0 | 1·76 | +9·4 |
| Popliteus ... ... | 0·52 | + 7·2 | 0·56 | + 7·5 | 0·50 | – 0·6 | 0·54 | +9·3 |
| Tibialis anticus ... ... | 1·29 | +18·2 | 1·14 | +24·3 | 1·10 | ?+ 5·0* | 1·16 | –0·3 |
| Extensor dig. com. ... | 1·30 | — | 1·68 | +18·6 | 1·60 | ?+ 5·0* | 1·47 | –7·5 |
| Peronei ... ... ... | 1·02 | +17·8 | 1·16 | +22·0 | 1·09 | +29·5 | 1·08 | +2·3 |
| | | | | Specific Gravity | | | | |
| Tibialis anticus ... ... | — | — | — | — | 1069 | 1067 | — | — |
| Extensor dig. com. ... | — | — | 1079 | 1073 | — | — | 1069 | 1069 |
| Peronei ... ... ... | — | — | 1070 | 1068 | 1053 | 1059 | — | — |

* The adhesions prevented accurate weighing.

It is we think clear that in these experiments the prolonged extension of the ankle, up to a certain stage of its action, decreased the loss of weight of the extensor muscles. Apparently it first caused increase of connective tissue at the joint, then around the tendons of the extensor muscles and to a varying extent in the intermuscular tissue. The comparative absence of effect on the tibialis anticus and extensor dig. com. in Exp. 3 we attribute to the encroachment of the connective tissue on the muscle substance which occurred in this case; this would tend to increase the rate of atrophy of the superficial fibres. The direct effect of extension on the muscle fibres cannot however be distinguished from the indirect effect of inflammation. It will be noticed that the greater weight of the extended muscles cannot be referred to absorption of water, for in Exp. 2 the difference in the specific gravity of the muscles was insufficient to account for the difference in weight, and in Exp. 3 the stretched peronei were of greater specific gravity than the relaxed. Nor does increase of connective tissue afford an adequate explanation, for the peronei presented to the eye practically no difference on the two sides.

In Exp. 1 there is apparently some lessening of the atrophy in the flexor muscles; in this case there was congestion of the vessels of the posterior surface of the leg, *i.e.* the result was probably due to slight inflammation.

The protracted flexion of the ankle in Exp. 4 had no very definite effect. But the muscles in which there was the greatest difference of weight on the two sides were the flexors and extensors of the toes (the popliteus can be disregarded on account of its small absolute weight). The flexors of the toes were more or less stretched and the extensor relaxed, so that the tendency of the result is to show that extension delayed the loss of weight.

### (c) *The effect of section of the tendons.*

The denervated gastrocnemius, plantaris and soleus are to a certain extent stretched in the normal sitting posture of the rabbit, and we thought we might determine the effect of complete relaxation on muscle atrophy by cutting the Achilles tendon. We made three experiments in which the sciatic nerve was cut on both sides and the Achilles tendon on one side. Further, since the gastrocnemius and plantaris muscles show in most cases a fairly constant loss of weight after about three weeks denervation, we made two experiments on these muscles by the unilateral denervation method, and for comparison one in which the tendons of the tibialis anticus and extensor dig. com. were cut. But the tendon section set up pathological conditions which made it impossible to distinguish the effect of relaxation of the muscle. There was more or less congestion of the blood vessels lying over the gastrocnemius, rapid growth of connective tissue around and between the cut ends of the tendon, and in some cases obvious changes in the appearance of the muscles. In all cases a rod of firm connective tissue joined the ends of the tendon, and this occurred even when the section was only of 12 days duration; in consequence on flexion of the foot, although the thigh was completely flexed, there was slight extension of the muscles; the extension naturally was less when a piece of the tendon was excised than when it was simply cut. The tendons spreading over the muscles attached to the Achilles tendon were more conspicuous on the cut side and more or less thickened.

We may take first the bilateral denervation experiments.

*Exp.* 1. Achilles tendon cut. Animal killed after 12 days. The muscles did not differ in appearance on the two sides except that the tendons in their course over the muscles were more conspicuous on the cut side. Some congestion of vessels of skin covering gastrocnemius.

*Exp.* 2. 0·75 cm. of Achilles tendon excised. Animal killed after 21 days. The weight of the body had been borne chiefly on the outer part of the heel, so that the heel was somewhat twisted. The tendons spreading over the gastrocnemius, plantaris and soleus were distinctly thicker than on the excised side. A small hard lump had formed in the lower part of the plantaris.

*Exp.* 3. 0·75 cm. of Achilles tendon excised and the ligaments attached to it cut. Animal anæsthetised and killed after 23 days. Complete flexion of the foot (the thigh being also flexed) extended the gastrocnemius but did not quite stop the fibrillation in it. The conspicuousness of the tendons spreading over the muscles, and the twisting of the heel, were greater than in Exp. 2. The median deep part of the gastrocnemius had a faint yellowish tinge, differing from the pinkish tint of the rest of the muscle. There were some adhesions to parts of all the muscles attached to the cut tendons.

The central end of the Achilles tendon was in these cases cut off at its junction with the muscle, and not weighed with the muscle.

We have seen reason to believe from the results in the previous sections (*a* and *b*) that an inflammatory condition is accompanied in its early stage by lessened loss of muscle weight, and that if the inflammation progresses so far as to cause adhesions, it leads to an increased loss of muscle weight. The experiments on the effect of cutting the tendon tend to support these conclusions. In Exp. 1 it is true that the short period of denervation allows of a large experimental error, but it seems likely that the heavier weight of the gastrocnemius and plantaris on the side with cut tendon was in part due to the concurrent slight inflammation. In Exp. 2 there was adhesion of connective tissue

TABLE VIII. Section of sciatic on both sides and of Achilles tendon on left side. The percentage differences of the muscles with cut tendon are in black type.

| Exp. ... ... ... | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Duration of denervation | 12 days | | 21 days | | 23 days | |
| Weight at operation ... | 2·54 | | 1·75 | | 1·68 | |
| „ „ death... ... | 2·41 | | 1·85 | | 1·96 | |
| „ less gut ... ... | — | | 1·39 | | 1·41 | |
| | rt | p.c. diff. lf. | rt | p.c. diff. lf. | rt | p.c. diff. lf. |
| Gastrocnemius + plantaris | 8·02 | **+10·3** | 4·33 | **+ 0·5** | 4·13 | **− 8·4†** |
| Gastrocnemius ... ... | — | — | 2·97 | **− 4·9** | 2·66 | **− 8·2†** |
| Plantaris ... ... ... | — | — | 1·37 | **+10·9*** | 1·46 | **− 8·9†** |
| Soleus ... ... ... | 1·23 | + 0·7 | 0·68 | **− 8·3†** | 0·77 | **− 9·1†** |
| Flexor dig. com.... ... | 2·38 | − 4·7 | 1·63 | − 3·4 | 1·67 | + 7·2 |
| Popliteus... ... ... | 0·62 | − 0·3 | 0·52 | + 1·5 | 0·47 | + 2·3 |
| Tibialis anticus ... ... | 1·97 | − 9·1 | 1·03 | + 5·1 | 0·96 | + 1·9 |
| Extensor dig. com. ... | 2·30 | − 4·2 | 1·27 | − 0·2 | 1·50 | − 1·5 |
| Peronei ... ... ... | 1·55 | + 4·4 | 0·97 | + 6·2 | 0·99 | +10·2 |
| | | | Specific Gravity | | | |
| Flexor com. dig.... ... | — | — | — | — | 1074 | 1073 |
| Peronei ... ... ... | — | — | — | — | 1067 | 1069 |

* Connective tissue mass in lower end.

† The maximal difference, since the adhesions were cut away before weighing.

to the lower part of the soleus, and in Exp. 3 there were adhesions in the lower part of all the muscles connected with the cut tendon. Each of these was lighter than on the opposite side, with intact tendon. Since the adherent tissue was cut away before weighing the muscle, and this may have contained some muscle fibres, the percentage difference given in the table is the maximal difference, the actual difference may have been less, especially in the soleus. The greater weight of the plantaris in Exp. 2 may be fairly attributed to the round mass of connective tissue embedded in its lower end, found both by dissection and by microscopical examination. If this interpretation of the results is correct, it leaves little room for an effect by the additional relaxation of the muscles; in any case the results do not tend to show that relaxation lessens the atrophy.

The muscle with uncut tendons presented no difference to the eye, and the percentage differences of the weights on the two sides were for the most part well within the limits of simple bilateral denervation; so far as they depart from it, it is in the direction of the more stretched muscles having the greater weight.

In the unilateral denervation experiments the pathological changes resulting from tendon section were less than in Exps. 2 and 3 just mentioned. This was probably due to the tendon having been cut instead of having a portion excised. There was however considerable connective tissue growth around the rod of tissue connecting the cut ends, and the tendons in their course over the muscles were thicker than normal. The muscular tissue presented the usual appearance of denervated muscle at this stage.

*Exps.* 1 *and* 2. Left sciatic cut in middle of thigh, and Achilles tendon cut. In Exp. 1 the tendon of the left flexor dig. com. was somewhat affected and was larger than on the opposite side; the excess was cut off before weighing. In Exp. 2 there was a similar but less change in the flexor tendon.

*Exp.* 3. Left sciatic cut in middle of thigh, and lower tendons of tibialis anticus and extensor dig. com. cut. A sore formed on the left heel causing slight enlargement of the lower part of the Achilles tendon.

In all three experiments, the Achilles tendon was cut off before weighing the muscles.

TABLE IX. Unilateral section of sciatic nerve with section of tendon.

The muscles the tendons of which were cut are in black type.

| Exp. ... ... ... | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Duration of denervation | 21 days | | 23 days | | 21 days | |
| Weight at operation ... | 2·36 | | 1·76 | | 2·39 | |
| „ „ death... ... | 2·43 | | 2·17 | | 2·68 | |
| „ less gut ... ... | 1·94 | | 1·72 | | 1·99 | |
| | rt | p.c. diff. lf. | rt | p.c. diff. lf. | rt | p.c. diff. lf. |
| Gastrocnemius + plantaris | 13·38 | **−49·1** | 11·10 | **−49·9** | 14·16 | −43·8 |
| Gastrocnemius ... ... | 9·46 | **−51·8** | 8·06 | **−52·4** | 9·86 | −44·7 |
| Plantaris ... ... ... | 3·92 | **−42·4** | 3·03 | **−42·0** | 4·30 | −41·8 |
| Soleus ... ... ... | 1·44 | −31·7 | 1·30 | **−36·9** | 1·59 | −37·4 |
| Flexor dig. com... ... | 3·33 | −28·2 | 2·71 | −21·4 | 3·70 | −27·6 |
| Popliteus ... ... ... | 1·01 | −31·8 | 0·84 | −45·4 | 1·00 | −36·2 |
| Tibialis anticus ... ... | 2·74 | −32·1 | 2·22 | −31·7 | 2·64 | **−34·0** |
| Extensor dig. com. ... | 2·51 | −21·2 | 2·14 | −10·0 | 2·78 | **−26·4** |
| Peronei ... ... ... | 2·16 | −37·4 | 1·81 | −34·1 | 2·14 | −36·0 |

### 4. *Bilateral denervation experiments as criteria of the effect of treatment.*

In the foregoing sections the various experimental errors which have to be borne in mind in considering the effect of treatment on atrophy have been referred to either directly or incidentally. We may summarise these and add some remarks on the extent to which the percentage difference at the end of the period of denervation shows the relative rate of atrophy on the two sides according to the extent of the atrophy.

(*a*) The first source of error which presents itself is the inequality in the initial weight of the corresponding muscles. The percentage differences in weight of 153 muscles, or small groups of muscles, are given in Table II. These serve as a basis for estimating the degree to which differences of initial weight must be taken into account in considering the effect of treatment. The number of observations is not however sufficient to exclude the possibility of wider variations in individual cases.

(*b*) The difference in initial weight on the two sides differs in the several muscles, so that a difference of weight after unilateral treatment which gives a high degree of beneficial effect on one muscle may give little probability of such effect on another. The difference in initial weight is partly due to the difficulty of equal dissection on the two sides, especially when the muscles have to be scraped from bone. Since the error in dissection makes a greater variation in the percentage difference the less the weight of the muscle, the percentage difference of such muscles as the soleus and popliteus is open to greater experimental error, so far as this point is concerned, than that of the gastrocnemius or tibialis anticus.

(*c*) When the sciatic nerve, or either of its main branches, is cut in rabbits, trophic disturbances frequently occur, and these are apt to cause an unequal rate of loss of muscle substance on the two sides. If there is any accessory pathological condition on either side, the results are more or less untrustworthy as far as the effect of treatment is concerned. Accessory pathological conditions may be present initially, and in consequence it is necessary to examine muscles, tendons and bones for abnormalities. Since obvious abnormalities sometimes occur, it seems likely that a difference in initial weight should occasionally occur as a result of minor injuries in early life which have left no post-mortem sign. Differences due to this cause may be taken as included in our control experiments on 17 normal animals and on six with bilaterally denervated muscles, but an occasional wider difference than has been found cannot be excluded.

(*d*) Another possible source of error is that there may be a difference in the rate of atrophy due to an intrinsic difference in corresponding muscles. We have compared the weights on the two sides of 23 denervated muscles of six rabbits; the results are given in Table IV. Allowing in these some variation on the two sides due to slight trophic changes, we conclude from them and from other results that the difference in the rate of atrophy in corresponding muscles due to intrinsic differences is small, probably not exceeding in general 1 to 1·5 p.c., and in consequence that, when there is no trophic disturbance, the difference in weight at the end of bilateral denervation is due to the initial difference, with an error in general not exceeding 1 to 1·5 p.c.

(*e*) Taking the initial difference in weight on the two sides as the main source of error in estimating the relative atrophy in the absence of accessory pathological change, it is important to form some idea of the extent of the possible error with different degrees of atrophy. Since the initial weight of corresponding muscles rarely differs by more than 6 p.c., we give the particular case of a difference of ± 6 p.c. in initial weight, and a 6 p.c. greater final weight on the side subjected to treatment. Fig. 2 shows the less percentage atrophy on the treated side with varying degrees of atrophy apart from treatment.

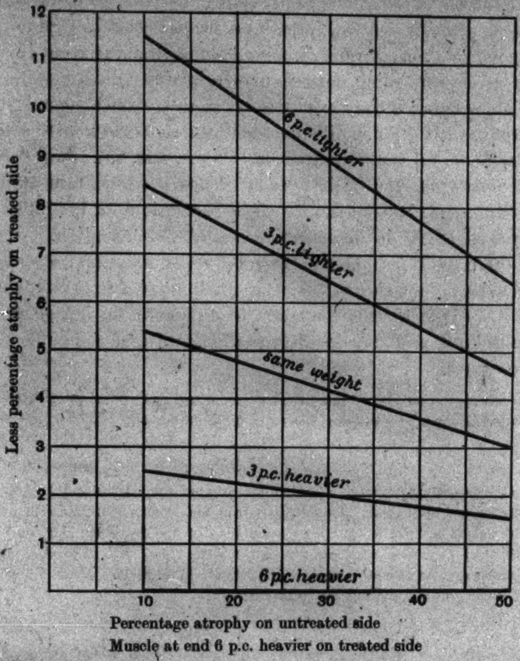


Fig. 2. Bilaterally denervated muscle. Less percentage atrophy on the treated side corresponding to initial greater weight of +6 to −6 on this side, and to a 6 p.c. greater weight at the end of the experiment.

It will be seen that when the atrophy apart from treatment is 50 p.c., the atrophy, with a + 6 p.c. final difference on the treated side, may be the same or 6·4 p.c. less than on the non-treated side. In nearly all our experiments the atrophy apart from treatment was 30 to nearly 50 p.c. Since the initial difference in weight was less than 6 p.c. in 92 p.c. of the cases, it does not require many experiments to enable conclusions to be drawn with a high degree of probability, if no pathological conditions occur, and if, as we think, the variation in rate of atrophy in corresponding muscles due to intrinsic differences is small. On the other hand, if the atrophy apart from treatment is only 10 p.c., the atrophy, with + 6 p.c. difference at the end on the treated side, may be the same as on the untreated or 11·5 p.c. less, *i.e.* there may be a gain of 1·5 p.c. on the initial weight. Thus a considerable final percentage difference is necessary to allow conclusions to be drawn if the atrophy is slight, *i.e.* if the denervation is of short duration.

(*f*) In the 17 normal animals, the flexor muscles were about as often heavier on one side as the other, and not to any greatly different extent. Consequently the error due to differences of initial weight in these muscles is probably small in the mean of a considerable number of observations.

If the initial weights are the same the less percentage loss ($P$) on the treated than on the untreated side can be readily calculated from the formula

$$P = p - \frac{a}{100} p,$$

where $p$ = the percentage greater weight of the treated side at the end of denervation, $a$ = the percentage total loss of weight apart from treatment. Thus if $a=30$ and $p=10$, the loss of weight is 7 p.c. less on the treated than on the untreated side, and if $a=50$ and $p=10$, the loss of weight is 5 p.c. less on the treated side.

The following more general formula we owe to Mr A. V. Hill.

Let $T$ be the initial weight of the treated muscle.

" $U$ " " " " untreated muscle.

Assume $T$ is $\beta$ p.c. greater than $U$, then

$$T = U\left(1 + \frac{\beta}{100}\right).$$

The final weight on the untreated side is $U\left(1 - \frac{a}{100}\right)$, and

" " " treated side is $T\left(1 - \frac{a-P}{100}\right)$.

The latter is $p$ p.c. greater than the former, so

$$T\left(1 - \frac{a-P}{100}\right) = U\left(1 - \frac{a}{100}\right)\left(1 + \frac{p}{100}\right),$$

or

$$U\left(1 + \frac{\beta}{100}\right)\left(1 - \frac{a-P}{100}\right) = U\left(1 - \frac{a}{100}\right)\left(1 + \frac{p}{100}\right).$$

i.e. $$P\left(1+\frac{\beta}{100}\right)=(p-\beta)\left(1-\frac{a}{100}\right).$$

As an approximation this can be simplified to

$$P=(p-\beta)\left(1-\frac{(a+\beta)}{100}\right),$$

or to include the case of $T$ being $\beta$ p.c. less than $U$,

$$P=(p\pm\beta)\left(1-\frac{(a\mp\beta)}{100}\right):$$

if the initial weights of the muscles are the same, this becomes

$$P=p\left(1-\frac{a}{100}\right),$$

as at the beginning of this paragraph.

(*g*) In each of the 17 normal animals taken, some of the muscles were heavier on one side, and some on the other side. Consequently if the muscles of the treated side are all heavier than on the other, and there has been no accessory pathological change, there is a probability that the treatment has delayed the atrophy in one or more of the muscles.

(*h*) A single experiment involving all the muscles of the leg, in which an apparently negative result is obtained, affords better evidence that the treatment has had little or no beneficial effect, than a similar experiment with an apparently positive result affords of a beneficial effect. The result obtained may be due to the initial weight of the muscles on the treated side being respectively lighter or heavier than on the other side, but it is only special cases of initial lighter weight that would allow the treatment to bring all the muscles into the normal limits, whereas every case of initial heavier weight would simulate a beneficial effect. A similar argument applies to the results of treatment accompanied by pathological symptoms.

(*i*) If the final weight of a treated muscle is considerably greater than that of the untreated, but the untreated is distinctly lighter in relation to the body weight than is usual in denervated muscle, there is some probability that the greater weight of the treated muscle is due to an abnormality of the untreated and not to a beneficial effect of the treatment.

### 5. *The effect of some forms of treatment.*

The theory that the muscle atrophy following nerve severance is due to the state of inaction of the muscle, and that the atrophy can be prevented by causing it to contract, was first put forward by John Reid in 1841 (4). His theory was based on experiments on frogs. He cut on both sides in four frogs the spinal nerve roots supplying the leg, and exercised the limb of one side daily for two months, by means of a galvanic current. He found that the muscles of the exercised limb retained their original size and contracted vigorously, whilst those of the opposite shrunk to half their size, and contracted feebly.

Reid's results led Brown-Séquard(5) to make similar experiments on rabbits. He said that frogs' nerves kept their irritability after section of the nerve roots, so that the effect obtained by stimulating the leg in the frog might be due to stimulation of the nerves.

It was shown later that in the frog the nerves keep their irritability for three to six weeks, and that the muscles do not lose weight on denervation at the rate described by Reid.

Brown-Séquard cut the sciatic nerve on both sides and stimulated one leg for six weeks. He stated that the muscles at the end of the time were normal on the stimulated side and notably atrophied and very slightly contractile on the other. Further he cut the sciatic on one side, and two months later when the muscles were atrophied, began stimulation and continued it daily for six weeks, at the end of which time he said the limbs were approximately of the same weight, and the exposed muscles on the two sides equally irritable. He considered that the galvanic current, besides keeping up the muscle weight by contraction, might have some direct action on the chemical change in the muscle similar to that produced by the nervous agent.

Brown-Séquard in a paper in the Journal de Physiol. 2, p. 75, 1859 reproducing some of his earlier papers referred to confirmatory experiments published by him in Experimental Researches applied to Physiology. New York, 1853. This work we have not seen.

The views of Reid and Brown-Séquard were not out of harmony with the facts known at the time, and in consequence the inactivity theory of atrophy—or as it was called later the disuse theory—obtained wide acceptance. In man, the production of contraction in paralysed muscles was not found to prevent atrophy, but it was generally thought to delay it. The theory was contested by many on the ground that the rate of loss of weight and the changes in the muscle after nerve section were of a different order from those occurring in disuse of the muscles arising from cerebral paralysis and from certain lesions of the spinal cord. On the other hand it was argued that in these cases reflex and tonic contraction were not abolished, and the disuse only partial. Thus whilst, in general, disuse was held to account for a part only of the atrophy the inactivity theory still had supporters. The theory put forward in its place, viz. that the atrophy is due to the absence of a trophic influence of the nerves, has as yet no definite meaning.

The experiments of **Paschutin** and others showing that there was very little lymph flow in normal quiescent muscle, and that the flow was increased by passive movement, and the experiments of **Lauder Brunton** and **Tunnicliffe** showing that massage of normal muscle increased the blood flow through it, together with increasing knowledge of the injurious action of metabolic products, seemed to afford a scientific basis for the employment of passive movement and massage in cases of nerve severance in man, for it might fairly be assumed that by these means blood and lymph flow would be increased in denervated as in normal muscle, and that growth would be promoted in one case as in the other. An experiment on the effect of each of these methods on the atrophy has been made by **Langley**(1) on the bilaterally denervated leg muscles of the rabbit. In one experiment the muscles of one side were made to contract a large number of times daily, and on the other there were repeated passive movements. In the former there was no indication of a delay in atrophy; in the latter there was some indication of a delay but not a certain effect.

Recently a different theory has been suggested by **Langley**(1), viz. that the atrophy is due to the continuous fibrillation which occurs in denervated muscle. Since **Langley** and **Kato** had found that calcium chloride in sufficient amount stopped the fibrillation, the former(3) made some experiments on the effect of giving calcium lactate with the food. No definite result was obtained either on the fibrillation or the atrophy.

Most of our experiments were made with an eye to this theory. It seemed possible that ionisation would be more effective than feeding in introducing into the muscle the quantity of calcium ion necessary to stop fibrillation. We first tried this on the exposed muscle. The foot was wrapped round with four or five folds of rather open meshed towelling moistened with Ringer's fluid, a plate electrode fastened over this, and connected with the negative pole of Watson's ionisation apparatus. The positive pole, sometimes a round flat electrode three quarters of an inch in diameter, sometimes a small plate electrode, was placed on similar towelling lying direct on the fibrillating muscle and moistened with 1 or 2 p.c. calcium chloride. With a current of 20 to 45 milliampères the fibrillation under the electrodes was stopped in 20 to 45 minutes. The minimal current and time required we did not determine. In parts of the muscle between the electrodes, there was usually slowing of the fibrillation but it was not quite stopped.

The ionisation did not in any case obviously affect the fibrillation in the deep surface of the muscle. The effect of ionisation through the skin was then tried; the skin was cut at the side of the leg, the fibrillation in the muscles observed, the skin sewn up and ionisation carried on. In this case the effect on the fibrillation was distinctly less, but in three quarters of an hour with a 25 m.a. current, fibrillation was stopped on the surface under the centre of the electrodes. Similar but weaker effects were obtained on ionisation with potassium chloride. These experiments indicated that to stop fibrillation in the deeper parts of the muscle would require stronger currents than the skin would bear. But since they showed that the fibrillation was sometimes stopped in the superficial layers by a current which was at any rate not painful it seemed worth while to try whether it had any effect on the muscle atrophy, and at the least the experiment would give some indication of the action, if any, of the galvanic current.

We may mention that in one of the experiments on ionisation with Ca salt which on account of pathological changes we thought would be useless for the purposes of the experiment (Exp. 1, Table X) we tried the effect of a strong current, 75–80 m.a., for three quarters of an hour through the muscles of the non-ionised side in the anæsthetised animal, using 1 p.c. CaCl at the + electrode. The heating effect was considerable and cold fluid (1 p.c. $CaCl_2$ solution and Ringer's fluid) had to be constantly dripping on the bandages lying between the electrodes and the skin. In this case the fibrillation was stopped on the deep surfaces of all the muscles as well as on the superficial surfaces.

In all the experiments on the effect of treatment the sciatic nerves were cut in the middle of the thigh, and in some, the internal saphenous nerve in addition.

In the experiments on the effect of ionisation on atrophy the protecting bandages were wrapped round the leg and round the foot. Flexible plate electrodes, tied on the bandages, were used; the + electrode was about 2¼ × 2¾ inches, it was placed on the anterior two thirds of the leg, the binding screw of the electrode being a little to the outer side of the leg, over the middle of the tibialis anticus; thus the greater part of the current passed through the extensor muscles. The – electrode was about twice the size of the + and encircled the foot. Carbon electrodes shaped for our needs we were unable to obtain. The rabbits were gently held and rarely moved during the passage of the galvanic current. Trophic changes in the skin caused by the current were in nearly all cases troublesome. Experiments were made with

1 to 2 p.c. $CaCl_2$, with Ringer's fluid, and with 1 p.c. KCl, at the positive pole.

### (*a*) *Ionisation with calcium chloride.*

The calcium salt caused in four to five days small lumps to form in the skin and it was impracticable to continue daily ionisation; after death these lumps showed as white patches on the under surface of the skin. The sub-cutaneous tissue was thickened especially in the regions of the lumps. A few white patches of lime salts of varying size were present in superficial part of the sheaths of the muscles, the maximum area of a patch was about 0·75 cm. In one case a few small white patches were present on the surface of the tibialis anticus; none were found in the interior of any muscle. In Exp. 1 fresh sub-cutaneous tissue was examined in serum, the elastic fibres, and these alone, were for the most part very refractive and calcified. In Exp. 2 the lumps in the skin were apparently due to proliferation of the cells of the hair bulbs, they were chiefly formed of finely fibrillated strands of varying length.

*Exp.* 1. Right leg, 14 days denervation. Ionisation begun on fourth day for 20 minutes twice a day. 20 milliampères for 5½ days. Lumps on skin over middle third of tibialis anticus; 15 m.a. for 2½ days. Small sore at lower edge of area with lumps. Round flat electrode on upper part tibialis anticus, 20 m.a., thickening of skin under electrode. Animal anæsthetised; non-treated leg ionised with $CaCl_2$, 75—80 m.a. for 45 minutes, current chiefly through gastrocnemius and plantaris, fibrillation stopped in deep surfaces of the muscles. On treated side, adhesions of the increased connective tissue to extensor tendons.

*Exp.* 2. Left leg, 20 days denervation. Ionisation on 4th day for 45 minutes once a day; 20 m.a. for four days; a few lumps on skin and antero-external part. For three days, ionisation on alternate days; lumps still increase; 15—18 m.a. with round flat electrode over upper part region of gastrocnemius for six days; skin here thickens. Animal anæsthetised, muscles look alike on the two sides except that the tibialis anticus and extensor dig. com. have some short red lines amongst the surface fibres as of hæmorrhage and that the left Achilles tendon is somewhat larger on the left than on the right; no discernible change in connective tissue of the muscles; left tib. ant. and ext. dig. com. more excitable on left than on right both to single induction shocks and to the galvanic current.

*Exp.* 3. Left leg, 23 days denervation. Ionisation on the third day, 30 m.a. 45 minutes once a day. In about a week the antero-external skin became scurfy and small lumps formed in it. After 12 days Ringer's fluid was substituted for 1 p.c. $CaCl_2$, for the rest of the time the skin remained in much the same condition. Post-mortem it was found that jelly-like connective tissue had grown from sores on the heel about a centimetre upwards between the tendons of the plantaris and gastrocnemius; less on the right than on the left. There was a trifling amount of jelly-like tissue between the muscles of the left side, but on both sides the muscles were healthy to the eye.

The pathological change which occurred in the skin in these experiments makes it hazardous to draw any conclusions from them as to the effect of ionisation on the muscle atrophy, but since they serve as a

Table X. Bilateral denervation. Ionisation of one side, mainly extensor muscles, with calcium salt, and with calcium salt followed by Ringer's fluid. The percentage difference given is that of the ionised leg.

| Exp. ... ... ... | 1 | | 2 | | 3 | | |
|---|---|---|---|---|---|---|---|
| Duration of denervation | 14 days | | 20 days | | 23 days | | |
| Ionisation ... ... | 20 mins. twice a day, 20 m.a. for 8 days, 15 m.a. for 2¼ days | | 15 to 20 m.a. 45 mins. for 12 days | | 30 m.a. 45 mins. CaCl$_2$ for 12 days; Ringer for 8 days | | |
| Weight at operation ... | 1·87 | | 2·03 | | 1·62 | | |
| „ „ death... ... | 1·96 | | 2·02 | | 1·68 | | |
| „ less gut ... ... | 1·38 | | 1·61 | | 1·24 | | |
| | lf. | p.c. diff. rt | rt | p.c. diff. lf. | rt | lf. | p.c. diff. lf. |
| Gastrocnemius + plantaris | 6·68* | − 7·6 | 6·56 | + 3·1 | 4·11 | 4·49 | + 9·1 |
| Gastrocnemius ... ... | 4·56 | − 4·4 | 4·40 | + 1·9 | 2·90 | 3·14 | + 8·3 |
| Plantaris ... ... ... | 2·13 | −16·4 | 2·15 | + 5·0 | 1·20 | 1·34 | +11·7 |
| Soleus ... ... ... | 0·83 | + 5·5 | 1·01 | + 4·3 | 0·57 | 0·68 | +16·6 |
| Flexor dig. com. ... | 1·67 | + 9·4 | 2·50 | + 5·9 | 1·23 | 1·30 | + 5·5 |
| Popliteus ... ... ... | 0·47 | −10·7 | 0·58 | − 0·3 | 0·41 | 0·45 | +11·1 |
| Tibialis anticus ... ... | 1·38 | +11·5 | 1·41 | + 6·3 | 0·85 | 1·24 | +46·2 |
| Extensor dig. com. ... | 1·80 | + 6·3 | 2·04 | −15·2 | 1·01 | 1·33 | +32·1 |
| Peronei ... ... ... | 1·06 | + 7·8 | 1·14 | + 1·1 | 0·88 | 0·94 | + 6·4 |
| | | | Specific Gravity | | | | |
| Plantaris ... ... ... | 1071 | 1069 | — | — | — | — | |
| Extensor dig. com. ... | — | — | 1073 | 1076 | 1072 | 1068 | |
| Peronei ... ... ... | 1077 | 1083 | — | — | — | — | |

* 75—80 m.a. for 45 mins. through left leg, chiefly flexors, just before death.

basis of comparison with any future similar experiments, we make some comments on the points of difference in the results.

Exp. 1 (Table X) we cut short and discarded on account of the condition of the skin; we utilised it, as mentioned above, to observe the effect of a strong current on fibrillation on the non-ionised side. The current passed chiefly through the gastrocnemius and plantaris, it is possible this caused a lessening of weight of these muscles. In Exp. 2 the skin was also affected, though less than in the preceding case. In the muscles themselves, there was no obvious pathological change, except some in the tibialis anticus and extensor dig. com., the muscles other than these were on the whole slightly heavier on the ionised side, but this may have been the result of the congestion of the skin vessels. Exp. 3 at first sight seems to show that ionisation retarded the atrophy, for the muscles on both sides appeared healthy to the eye. But if the weight of the muscles on the stimulated side is compared with that of the muscles in a normal animal of about the same weight, *e.g.* with those of Exp. 14, Table II, it is clear that their loss of weight was considerable, and apparently it was within the limits of loss which occurs in denervated muscle apart from treatment. And this suggests that the difference on the two sides may have been due to a difference existing before denervation. It is true that careful examination showed no pathological condition either in bones or muscles, and that apart from such conditions we have not found either in normal or denervated muscles differences in weight such as occurred in this experiment, but as we have said it is to be expected that considerable differences on the two sides will occasionally occur either arising from congenital defects or as a result of early injuries which in other respects have been recovered from. In consequence we are not inclined at present to lay stress on this experiment.

### (*b*) *Ionisation with Ringer's fluid.*

These experiments and also Exps. 1 and 2, Table XIII, were made with pure bred silver grey rabbits. They were more active in their movements than the rabbits of mixed breeds and in consequence after denervation, sores formed in all cases in the heel; this did not however cause any swelling of the Achilles tendon. As an indication of the extent of atrophy in the denervated muscles we give in Exp. A the muscle weights on the two sides in a rabbit of the same breed killed without any operation. The Ringer's fluid contained 0·04 p.c. calcium chloride. The ionisation caused lumps in the skin somewhat like those caused by calcium salts, inflammation and thickening of the sub-cutaneous tissue, and a few small white patches of lime salts in the tissue below; the trophic changes however were considerably less than with calcium salts.

*Exp.* 1. Left leg; 20 days denervation. Ionised with 30 m.a. for three quarters of an hour a day except on the first two days. There was a small white patch in the aponeurosis covering the tibialis anticus, and slight connective tissue growth around the lower tendon of the extensor dig. com.

*Exp.* 2. Left leg; 22 days denervation. Treatment as in Exp. 1 except that for the first four days the current was 20 m.a. for half an hour twice a day. On either side midway in the leg, the skin was thickened, and had some lumps in it. Anteriorly and posteriorly the skin was normal looking, but anteriorly on cutting it, it was found that it was somewhat inflamed and the sub-cutaneous tissue increased in amount. The aponeurosis covering the tibialis anticus was a little thickened but not the sheath of the muscle. The sub-cutaneous tissue where there were lumps externally had small white lumps but there were none deeper than this. All the muscles looked healthy.

TABLE XI. Exps. 1 and 2, bilateral denervation, ionisation on left side with Ringer's fluid. Exp. 3, undenervated animal. All pure bred silver grey rabbits.

| Exp. ... ... ... | 1 | | 2 | | A | |
|---|---|---|---|---|---|---|
| Duration of denervation | 20 days | | 22 days | | — | |
| Ionisation ... ... | 30 m.a. 45 mins. for 18 days | | 20 m.a. 45 mins. for 4 days, 30 m.a. 45 mins. for 15 days | | — | |
| Weight at operation ... | 1·67 | | 1·94 | | — | |
| " " death... ... | 1·63 | | 1·84 | | 1·80 | |
| " less gut ... ... | 1·30 | | 1·46 | | 1·46 | |
| | rt | p.c. diff. lf. | rt | p.c. diff. lf. | rt | p.c. diff. lf. |
| Gastrocnemius + plantaris | 5·35 | − 2·5 | 6·03 | +0·5 | 11·03 | −0·9 |
| Gastrocnemius ... ... | 3·65 | − 4·8 | 4·07 | +3·5 | 7·80 | +0·2 |
| Plantaris ... ... ... | 1·69 | + 2·0 | 1·95 | −5·6 | 3·45 | −3·7 |
| Soleus ... ... ... | 0·86 | − 0·4 | 0·79 | −4·3 | 1·06 | +0·9 |
| Flexor dig. com.... ... | 1·89 | − 3·9 | 2·33 | −5·2 | 3·08 | −2·7 |
| Popliteus ... ... ... | 0·46 | − 0·7 | 0·44 | +3·7 | 0·70 | +2·5 |
| Tibialis anticus ... ... | 1·16 | +11·5 | 1·38 | −1·6 | 2·29 | +3·9 |
| Extensor dig. com. ... | 1·67 | + 1·4 | 1·57 | −9·3 | 2·24 | −0·3 |
| Peronei ... ... ... | 1·03 | +10·4 | 1·26 | +5·9 | 1·83 | −5·8 |
| | | | Specific Gravity | | | |
| Tibialis anticus ... ... | 1063 | 1064 | — | — | | |
| Extensor dig. com. ... | — | — | 1075 | 1072 | | |
| Peronei ... ... ... | 1073 | 1068 | — | — | | |

In the foregoing experiments the weights of the flexor muscles on the two sides differed but little, and the differences are within the limits occurring in normal healthy rabbits, so that the galvanic current which passed through these muscles could have had very little if any effect upon them. The extensor muscles in three out of six cases had a difference of weight greater than that usually occurring in normal rabbits. These received a stronger galvanic current than the flexors but the results are not consistent, and since the sub-cutaneous tissue over them was thickened, the differences are more probably due to inflammatory conditions than to the direct action of the current. The weights of the muscles in a normal animal of the same breed are given in Exp. A. It will be seen that in all the denervated muscles of Exps. 1 and 2 there was large atrophy.

### (c) *Ionisation with a potassium salt.*

*Potassium chloride.* With this no lumps formed on the skin, but in two cases there were some trophic changes at the upper end of the negative electrode.

*Exp.* 1. Right side treated. 21 days denervation, 20 m.a. for half an hour twice a day, beginning after 3 days. The movement of the ankle became slightly stiffer and post-mortem slight connective tissue growth was found at the lower part of the tendons of the tibialis anticus and flexor dig. com.

*Exp.* 2. Left side treated. 22 days denervation, 30 m.a. for three quarters of an hour once a day, beginning after 3 days. A scab formed on the foot and the hairs came off in front of this and for a short distance on the dorsal surface. At death it was seen that the lower part of the extensor dig. com. was congested. The left tibialis ant. and extensor dig. com. were more irritable to the galvanic current than those of the right side.

*Exp.* 3. Right side treated. 23 days denervation, 20 m.a. for three quarters of an hour a day for 8 days, beginning after 5 days. Two days rest; 30 m.a. for three quarters of an hour a day for 8 days. At the end, in the anæsthetised animal the left tibialis ant. was slightly less irritable to single induction shocks, and distinctly more irritable to the galvanic current than the right. No difference in the irritability of the gastrocnemius or extensor dig. com. on the two sides to single induction shocks.

TABLE XII. Bilateral section of the sciatic. Ionisation of one side with potassium salt.

| Exp. ... ... ... | 1 | | 2 | | 3 | | |
|---|---|---|---|---|---|---|---|
| Duration of denervation | 21 days | | 22 days | | 23 days | | |
| Ionisation ... ... | 20 m.a. for 30 mins. twice a day for 18½ days | | 30 m.a. for 45 mins. for 19 days | | 20—30 m.a. for 45 mins. for 16 days | | |
| Weight at operation ... | 2·38 | | 2·31 | | 2·07 | | |
| „ „ death... ... | 1·94 | | 2·15 | | 2·11 | | |
| „ less gut ... ... | 1·62 | | 1·75 | | 1·77 | | |
| | lf. | p.c. diff. rt | rt | p.c. diff. lf. | lf. | rt | p.c. diff. rt |
| Gastrocnemius + plantaris | 6·09 | −1·8 | 6·18 | 0 | 7·50 | 8·16 | + 8·9 |
| Gastrocnemius ... ... | 4·07 | −2·8 | 4·15 | + 1·0 | 4·87 | 5·33 | + 9·4 |
| Plantaris ... ... ... | 2·01 | −0·5 | 2·01 | − 2·2 | 2·60 | 2·81 | + 8·1 |
| Soleus ... ... ... | 0·83 | +4·2 | 0·82 | + 1·2 | 0·99 | 1·10 | +10·9 |
| Flexor dig. com. ... | 2·33 | −0·3 | 2·00 | − 0·5 | 2·99 | 3·03 | + 1·4 |
| Popliteus ... ... ... | 0·62 | +0·7 | 0·48 | − 5·2 | 0·72 | 0·73 | + 2·1 |
| Tibialis anticus ... ... | 1·58 | +3·2 | 1·26 | + 6·8 | 1·42 | 1·87 | +30·8 |
| Extensor dig. com. ... | 1·72 | +8·0 | 1·76 | −10·5 | 1·66 | 1·76 | + 5·7 |
| Peronei ... ... ... | 1·13 | +3·8 | 1·07 | + 5·9 | 1·20 | 1·31 | + 8·9 |
| | | | Specific Gravity | | | | |
| Extensor dig. com. ... | — | — | 1070 | 1076 | 1074 | 1076 | — |

We made one other experiment. Ionisation with KCl on the left side, 30 m.a. 45 minutes a day, begun after 3 days; on the tenth day a scab formed a little above the ankle; on the evening of the eleventh day, the left ankle was swollen and had apparently been strained in movement. On the 12th day, as the foot was still swollen, and the leg slightly, the animal was killed. The left popliteal gland was enlarged. All the muscles on the left side were heavier than on the right. The percentage differences were: gastrocnemius +7·8, plantaris +11·7, soleus +13·7, flexor ped. com. +3·8, popliteal +2·3, tibialis anticus +8·9, extensor dig. com. +4·99, peroneus +8·4. Sp. Gr. flexor ped. com., Rt 1069, Lf. 1070, of tibialis anticus, Rt 1070, Lf. 1066. The results are in a line with those of the others in which there was inflammation accompanied by œdema.

In Exps. 1 and 2 (Table XI) the differences in the weight of the flexor muscles were well within the normal limits. We have seen that in the two experiments on ionisation with Ringer's fluid, the differences in the flexors were also in normal limits, though they were greater than those in Exps. 1 and 2 (Table XI). As we have said the effect of ionisation on the fibrillation, with the strength of current here used, was superficial. The bulk of the flexor muscles are not on the surface. We think then that in these experiments there was relatively little transference of ions into the muscles from the exterior into the greater part of the flexors and that the experiments differ but little from experiments on the simple passage of a galvanic current. If as seems probable the currents employed were as strong as could be safely sent through the skin of man, the results give fairly good evidence that the galvanic current as ordinarily employed does not affect the atrophy of denervated muscle.

The current was stronger and the ionisation greater in the extensor muscles. The general impression given by the results in these is that there was a slight beneficial effect, bearing in mind that the only ionised extensor muscle which had less weight was the only one which appeared abnormal to the eye. But there was some connective tissue growth, so that the apparent slight benefit may have been brought about by slight inflammation. The third experiment is more difficult to account for except on the theory that the ionisation retarded the atrophy, but in view of the fact that the gastrocnemius and plantaris were in this experiment heavier on the ionised side and were apparently uninfluenced in Exp. 2 we do not think any certain conclusion can be drawn.

### (*d*) *Rhythmic flexion and extension. Massage.*

One experiment has been published by Langley(3) of bilateral denervation in which the leg of one side was rhythmically flexed and extended for two and a half hours a day for 21½ days out of the 23 days of denervation. The flexors were affected little if at all, the extensors were all somewhat outside the normal limits of difference, but two of the three were lighter on the treated side than on the non-treated side. Since it seemed possible that the passive movement in this experiment was carried on for too long a period we tried the effect of similar flexion and extension (Exp. 1, Table XIII) for half an hour morning and evening. We also tried the effect of massage, but this at first was too strong for the delicate skin and caused some sores on the antero-lateral surface.

*Exp.* 1. Silver grey rabbit, pure breed, 21 days denervation. The left foot was taken in the hand and fully, but gently, extended and flexed rhythmically for half an hour morning and evening during the last 18 days. At death there was on both sides a small scab on the dorsal surface of the second and third toes, and these were somewhat swollen; the injury was rather greater on the left than on the right side. There were sores on both heels, and on the left side this had caused a slight connective tissue growth at the lower end of the Achilles tendon. The inter-muscular connective tissue was slightly greater on the left side.

*Exp.* 2. Silver grey rabbit, pure breed, 23 days denervation. Left leg, firm massage begun on third day, half an hour twice a day; this caused sores on the antero-lateral part of the skin, and after 4 days, massage was stopped for 4 days. More gentle massage for the next 11 days, chiefly of flexors, and the sores gradually healed. At death there were sores on both heels and increased connective tissue around both Achilles tendons. On the left side there was slight increase of jelly-like connective tissue on the surface of the flexor muscles; the tendons of the plantaris and flexor dig. com. on the surface of the muscles were more distinct on the left than on the right.

TABLE XIII. Bilateral denervation. Rhythmic extension and flexion. Massage. Left side.

| Exp. ... ... ... | 1 | | 2 | |
|---|---|---|---|---|
| Treatment ... ... | Rhythmic extension and flexion ½ hour twice a day for 18 days | | Massage ½ hour twice a day for 15 days | |
| Duration of denervation | 21 days | | 23 days | |
| Weight at operation ... | 2·17 | | 1·80 | |
| „ „ death... ... | 1·98 | | 1·85 | |
| „ less gut ... ... | 1·63 | | 1·47 | |
| | rt | p.c. diff. lf. | rt | p.c. diff. lf. |
| Gastrocnemius + plantaris | 7·26 | +3·9 | 6·83 | + 4·9 |
| Gastrocnemius ... ... | 4·83 | +0·9 | 4·60 | + 4·7 |
| Plantaris ... ... ... | 2·42 | +9·9 | 2·21 | + 5·2 |
| Soleus ... ... ... | 0·71 | +7·0 | 0·73 | +10·8 |
| Flexor dig. com.... ... | 2·33 | +7·7 | 2·36 | + 4·9 |
| Popliteus ... ... ... | 0·53 | +1·1 | 0·51 | + 1·4 |
| Tibialis anticus ... ... | 1·52 | −0·9 | 1·51 | + 5·6 |
| Extensor dig. com. ... | 2·07 | +6·6 | 1·84 | − 4·2 |
| Peronei ... ... ... | 1·29 | +1·5 | 1·29 | + 7·4 |
| | | Specific Gravity | | |
| Plantaris ... ... ... | 1070 | 1068 | — | — |
| Soleus ... ... ... | — | — | 1076 | 1071 |
| Tibialis anticus ... ... | 1067 | 1063 | — | — |
| Extensor dig. com. ... | 1069 | 1070 | 1062 | 1066 |

In these two experiments there is a slight balance in favour of the treated side. But in both of these there was slight connective growth on this side, and the conditions accompanying connective growth may have caused an increase of weight. At the best, the effect of the treatment on the atrophy was slight. In the massaged flexor muscles the change in the inter-muscular tissue, though slight, was distinct, and the possibility that massage, unless gentle, causes growth of connective tissue is one to be borne in mind.

It must be noticed that the un-massaged leg was not immobilised, and that the movements of the animal would cause some pressing on of lymph from the denervated muscles. If this causes any delay in atrophy, the experiment tends to show that slight massage at not infrequent intervals is sufficient to produce nearly the maximal, if not the maximal, effect possible.

## UNILATERAL DENERVATION.

Since we have considered above the experimental errors involved in the bilateral denervation method, we consider in the following section, for the sake of comparison, those involved in the unilateral method.

### 1. *The experimental errors in determining the effect of treatment in unilateral denervation.*

The determination of the effect of treatment depends on a comparison of the degree of atrophy in a given time in treated and untreated animals. The degree of atrophy in both cases is estimated from the relative weights of the denervated and undenervated muscles. In making the estimate the following points have to be taken into account.

The possible error due to initial difference of weight is large because it comes twice into the reckoning, once in the control animal and once in the treated animal. Thus to take a case similar to that we have taken in considering bilateral denervation, suppose that the initial difference of weight is ± 6 p.c. and that after unilateral denervation the percentage difference of weight is 30 p.c. in the control and 24 p.c. in the treated animal, the rate of atrophy being the same. Then if the initial percentage difference is of different sign in the two animals, the atrophy in the treated animal will either be 14·8 p.c. less than the control or 2·3 p.c. greater. The range of error will only be reduced by about 2 p.c. in each direction if the atrophy is 50 p.c. instead of 30 p.c. On the other hand if the muscles in the two animals are initially of the same weight, or if the percentage differences are the same and of the same sign, the atrophy in the treated muscle is approximately 6 p.c. less than in the control. So far as the error involved in the initial rate is concerned, the unilateral denervation method will give fairly trustworthy results in any muscle the weight of which on the two sides differs constantly by a small percentage only, and the chances are much in favour of an appreciably less loss in the treated than in the control animal being due to a beneficial effect of treatment though the degree of benefit is guess work.

A second point to be considered is the extent to which the rate of atrophy varies in the corresponding muscles of different animals. It is to be expected that some variation would occur in different breeds and in different states of health, especially in the state of health after the operation. Since a variation in the rate of atrophy would make the limits of difference in weight occurring in denervated and undenervated muscles in different experiments wider than if there were no such variation; its occurrence can be determined by comparing the limits due to initial difference with that found in experiment. A comparison on this basis we make later, it does not however seem possible to determine the variation in rate of atrophy with any exactness.

Further, if tension affects the atrophy, the varying duration of the positions of the hind legs will cause a different rate of atrophy in different animals. There are two other factors which may affect the accuracy of the estimate of the degree of atrophy. (*a*) There may be some variation in weight on the undenervated side in consequence of decreased or increased use. The former is the more probable, but since it would be operative both in the experimental and control animal, it is unlikely that the error due to this is more than slight. (*b*) In young animals, the undenervated muscles may continue to grow, and this would tend to make the observed difference in weight greater than the difference due to atrophy. The growth however is not likely to be important unless the treatment is prolonged.

It will be noticed that these possibilities of error in estimating the effect of treatment are either smaller or non-existent in bilateral denervation.

So far as constancy in the percentage difference of weight is concerned the gastrocnemius + plantaris appears to be much the most favourable group of muscles for experiment. In six out of eight cases (cp. Table XIV) the difference in weight on the two sides in these muscles taken together only ranged from 43·1 to 47·8 p.c. But the constancy is in part due to the fact that the two muscles frequently vary independently and thus a greater equality of difference in loss of weight obtained in different experiments when they are taken together than when they are taken separately. In one third of the cases of bilateral denervation in which the separate weights were taken, the percentage difference of the two muscles was of different sign, and this is rare in the normal state. Whilst the error due to dissection is to some extent the cause of this, it is we think clear that the conditions of the experiments affected the muscles unequally. It may be noted that in the two experiments of Table IX in which the tendo Achillis was cut, the percentage difference of the plantaris was much the same as in the other experiments on unilateral denervation (Table XIV) but that of the gastrocnemius was much greater.

We have not thought it worth while to make experiments on the effect of treatment by the unilateral denervation method, but it seemed desirable to make some observations by it with the object of determining whether there is any marked difference in the rate of atrophy of different muscles.

### 2. *Relative rate of atrophy in different muscles.*

It is known that the external sphincter of the anus—a striated muscle—atrophies but little after denervation. This muscle however differs so much from skeletal muscle in its properties that the fact cannot be treated as an argument in favour of a difference in the rate of atrophy of skeletal muscles. It has been said that after nerve section the facial muscles atrophy more slowly than those of the limbs. This affords a better basis for argument but still not a satisfactory one. Langley and Kato (2 p. 438) in explanation of the result of one of their experiments on the rabbit suggested that there might be a less atrophy of the tibialis anticus than of the gastrocnemius. Roberts(6) concludes from his experiments—made also on the rabbit—that the rate of atrophy of the tibialis anticus is constantly less than that of the gastrocnemius. In the cat, Langley(3) found a slower rate of atrophy in the soleus than in other muscles. It must be borne in mind that on account of the large possible experimental error, the degree of certainty of the conclusions depends upon the number of the experiments.

The results of our experiments are given in Table XIV. The nerves were cut on the left side. In two of these, viz. Exps. 3 and 7, there were some transient accessory pathological changes. We have seen that these are commonly accompanied by some variation beyond the normal limits; and, as it is important to correlate the two, we give notes on these cases.

*Exp.* 3. The cutaneous incision was much longer than in the other experiments, some of the sutures were torn out, and the skin healed slowly. Though there is nothing in the results on the flexor muscles to distinguish them from the cases in which the healing was rapid, it is possible that the inflammation helped to cause the greater percentage difference in the superficial extensors.

*Exp.* 7. In order to prevent injury to the dorsal surface of the toes, a bandage was

Table XIV. Comparative weight of leg muscles after denervation on one side (rabbit).

The percentage differences of the denervated muscles are in black type.

| Exp. ... ... | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of days denervation ... | 20 | | 21 | | 22 | | 21 | | 23 | | 25 | | 25 | | 21 | |
| Weight | | | | | | | | | | | | | | | | |
| at operation ... | 1·70 | | 1·91 | | — | | 2·12 | | 2·32 | | 2·03 | | 1·90 | | 1·92 | |
| at death ... | 1·92 | | 2·05 | | 2·83 | | 2·42 | | 2·75 | | 2·42 | | 2·06 | | 1·92 | |
| less gut ... | — | | — | | 2·27 | | 1·66 | | 2·14 | | 1·69 | | — | | 1·51 | |
| | Weight on right | p.c. diff. left | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. | Wt rt | p.c. diff. lf. |
| Gastrocnemius + plantaris ... | 10·61 | **−46·6** | 12·54 | **−47·8** | 17·07 | **−46·4** | 11·68 | **−44·2** | 15·32 | **−56·8** | 12·56 | **−38·6** | 10·01 | **−45·9** | 10·20 | **−43·1** |
| Gastrocnemius ... | — | — | — | — | — | — | 8·37 | **−44·2** | 10·79 | **−57·5** | 9·00 | **−38·7** | — | — | — | — |
| Plantaris ... ... | — | — | — | — | — | — | 3·30 | **−44·0** | 4·53 | **−55·1** | 3·57 | **−38·4** | — | — | — | — |
| Soleus ... ... ... | 1·04 | **−26·8** | 1·33 | **−28·6** | 1·86 | **−33·0** | 1·21 | **−38·4** | 1·39 | **−32·1** | 1·33 | **−30·3** | 1·31 | **−45·2** | 1·18 | **−25·1** |
| Flexor dig. com.... | 2·50 | **−30·0** | 2·91 | **−25·5** | 4·04 | **−31·8** | 2·59 | **−20·2** | 3·58 | **−41·0** | 3·17 | **−22·1** | 2·20 | **−16·8** | 2·83 | **−33·6** |
| Popliteal ... ... | 0·67 | **−37·3** | 0·76 | **−29·1** | 1·00 | **−46·1** | 0·99 | **−37·2** | 1·17 | **−42·1** | 0·90 | **−35·4** | 0·73 | **−38·9** | 0·78 | **−42·0** |
| Extensor dig. I ... | — | — | — | — | 0·69 | **−40·4** | 0·42 | **−21·2** | 0·54 | **−41·6** | 0·55 | **−34·7** | — | — | 0·43 | **−29·5** |
| Tibialis anticus ... | 1·81 | **− 4·2** | 2·19 | **− 2·7** | 3·20 | **−10·7** | 2·30 | **−27·2** | 2·90 | **−47·3** | 2·16 | **−44·0** | 2·06 | **−39·1** | 1·84 | **−21·2*** |
| Extensor dig. com. | 1·75 | **− 8·5** | 2·29 | **− 8·1** | 2·87 | **− 9·5** | 2·21 | **− 0·2** | 2·62 | **− 6·7** | 2·13 | **−20·6** | 2·03 | **−18·0** | 1·73 | **− 0·5*** |
| Peronei ... ... | 1·36 | **− 5·7** | 1·71 | **+ 3·5** | 2·36 | **− 0·4** | 1·66 | **−22·7** | 2·42 | **−51·2** | 1·90 | **−28·4** | 1·45 | **−21·2** | 1·40 | **−25·0*** |

* External popliteal nerve compressed, unequal degeneration in the nerves.

passed round the foot and leg so as to keep the foot flexed. This was successful at first, but later the bandage became tightened in the movements of the animal, and the foot and leg were found to be oedematous. The bandage was removed, and the oedema disappeared in the course of the second day. The results differ from those of the other observation in the greater difference of weight in the soleus muscles, and in the less difference of weight in the flexors dig. com.

The results given in Table XIV show, as was to be expected from the considerations we have given in the preceding section, that the difference in weight on the two sides varies widely in different animals after about three weeks denervation on one side, though in the majority of cases the gastrocnemius and plantaris have a fairly constant loss. The extent of the variation in different muscles is better seen by arranging the results as in Table XV. In this Table, the figures in black type refer to experiments on simple unilateral denervation (Table XIV and the extensors in Exp. 4, Table IV), those in ordinary type refer to experiments in which the Achilles tendon was cut (Table IX) and those in italics to those in which the tendons of the tibialis anticus and extensor dig. com. were cut (Table IX).

The arrangement of the results in Table XV shows at a glance that in the majority of cases the percentage difference in the weights of the denervated and undenervated gastrocnemius + plantaris muscles was greater, and the percentage difference of the extensor dig. com. was less, than that of the other muscles. It is we think clear that the difference cannot be explained by difference in initial weight, so that there must be some factor which causes a difference in the rate of atrophy. When the difference in the rate of atrophy is great, as between the gastrocnemius and plantaris, and the extensor dig. com., the percentage difference at death cannot be equalised by initial difference of weight, but as the difference in the rate of atrophy decreases the difference in initial weight will in particular cases tend to blur the effect of difference in rate, and the occasional approximate equality in the percentage difference of the gastrocnemius + plantaris and of the extensor dig. com. with some of the other muscles may be accounted for in this way.

The atrophy of the extensor dig. com. presents some striking facts. In Exp. 3 this muscle after denervation had nearly the same weight as the non-denervated, and in Exp. 4 it was only 6·7 p.c. lighter. The maximum difference in weight on the two sides which we have found in normal animals is about 5 p.c. If the denervated muscles were 5 p.c. heavier to start with, there would still be, so far as this is concerned, an atrophy of only 5 and 11 p.c. respectively in the experiments. We have given evidence that the muscles on the undenervated side lose weight,

. **Table XV.** Percentage difference in the weight of the muscles on the two sides 20 to 25 days after unilateral denervation.

| Percentage difference .. .. | 0–4·9 | 5–9·9 | 10–14·9 | 15–19·9 | 20–24·9 | 25–29·9 | 30–34·9 | 35–39·9 | 40–44·9 | 45–49·9 | 50–54·9 | 55–56·8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gastrocnemius + plantaris | — | — | — | — | — | — | — | 1 | 2. *1* | 4. 2 | 0 | 1 |
| Soleus ... ... ... | — | — | — | — | — | 3 | 3. 1 | 1. 1. *1* | 1 | — | — | — |
| Flexor dig. com. ... ... | — | — | — | 1 | 2. 1 | 2. 1. *1* | 1 | 1 | 1 | — | — | — |
| Popliteus ... ... ... | — | — | — | — | — | 1 | 1. 1 | 3. *1* | 2. 1 | 1 | — | — |
| Extensor dig. I ... ... | — | — | — | — | 1 | 1. *1* | 1. 1 | 0 | 2. 1 | — | — | — |
| Tibialis anticus ... ... | — | — | — | — | — | 1 | 2. *1* | 2. 1 | 1 | 1 | — | — |
| Extensor dig. com. ... | 1 | 1. 1 | 0 | 1 | 1. 1 | 1. *1* | — | — | — | — | — | — |
| Peronei ... ... ... | — | — | — | — | 2 | 1 | 0 | 1. 2. *1* | 0 | 0 | 1 | — |

Figures in black type refer to experiments in Table XIV (the extensor muscles of Exp. 8 are omitted since the denervation was not complete, those of Exp. 4, Table IV, are included).

Figures in ordinary type refer to the experiments in which the Achilles tendon was cut, those in italics to the experiments in which the tendons of the tibialis anticus and extensor dig. com. were cut (cp. Table IX).

but in both of these cases the ratio of the weight of the muscle to the body weight and to that of the other undenervated muscles was much as usual, so that the low percentage difference can hardly be attributed to loss on the undenervated side. And in Exp. 4, Table VI, in which there was bilateral denervation of the extensor dig. com., both muscles were nearly of the same weight as in normal animals. That in the conditions of the experiments the extensor dig. com. atrophies less than the other muscles is shown by a comparison of the relative weights of this muscle and of the tibialis anticus in normal and in denervated muscles. In the 15 normal animals (Table I and II) the two muscles differ but little in weight, but the advantage is somewhat on the side of the tibialis anticus. In 19 out of 20 cases after denervation the extensor dig. com. was heavier than the tibialis anticus. The cause of the variation in rate of atrophy we shall consider presently.

The results with the other muscles though they indicate some variation in the rate of atrophy hardly justify any definite conclusions.

On comparing the foregoing results on the rabbit with those obtained by Langley(3) in the cat, it appears that in the two animals after three weeks denervation the degree of atrophy in the gastrocnemius and plantaris is about the same, but in the rabbit these muscles atrophy in general more than any other muscle, while in the cat they are not differentiated from several others. Further in the rabbit the extensor dig. com. has in general the least rate of atrophy whilst in the cat this place is taken by the soleus.

It will be seen from Table XV that the differences in weight between the denervated and undenervated muscles in different rabbits lies within certain limits, and though these limits would no doubt be increased by additional observations, it is clear that any beneficial treatment carried out in a number of rabbits would show itself in a lower scale of differences.

There are two other aspects of the data to be noticed:

(*a*) The limit of the difference in weight of the denervated and undenervated muscles is in most cases rather more than 20 p.c.: whilst variations in initial weight might occasionally cause variations to this extent, the variations found in the weights of denervated and undenervated muscles are we think too great to be referred to this cause and we attribute them in part to a difference in the rate of atrophy of the muscles in different animals.

(*b*) In Exps. 1, 2, and 3 the only nerve cut was the internal popliteal nerve on the left side, but taking the three experiments together eight of the nine muscles with intact nerves were lighter on the left side than on the right. This proportion is much greater than in normal animals. A similar disproportion in similar experiments on the cat has been pointed out by one of us (3). The result may in part be put down to disuse wasting, since the muscles on the partially denervated side are for a time at any rate less used than on the other side. It is possible however that it is in part due to reflex nutritional disturbance, for nervous impulses both exciting and inhibitory are cut off from the extensor centres by section of the flexor nerves.

The differences in the rate of atrophy have no doubt more than one factor. Langley and Kato suggested that a less atrophy of the tibialis anticus than of the gastrocnemius might be caused either by a more frequent passive extension or a less natural rate. Roberts, on the ground that it was well known that stretched muscles do not recover after nerve suture, considered that the difference he found between the tibialis anticus and gastrocnemius was due to the former being relaxed and the latter being stretched. The premise is unsound, for moderate stretching of a muscle such as that of the gastrocnemius in these experiments does not prevent recovery. But the explanation of the difference in the rate of atrophy might be true. Though there are no experiments so far as we know, showing the effects of stretching a muscle on its metabolism, there would be nothing surprising if the sum of its effects were a loss of muscle substance. The question can however only be settled by experiment. The experiments of one of us (3) on the cat do not favour the theory that differences in the degree of tension are sufficient to account for the differences which occur in the extent of atrophy in different muscles. In each of six cases of unilateral denervation the estimated percentage loss was considerably less in the soleus than in the other muscles, and it is certain that the soleus could not have been the muscle which was stretched the least.

The direct experiments we have described above (p. 34) on the effect of tension on atrophy have not given a definite result, but they tend to show that with nearly complete relaxation there is at least as much atrophy as with moderate intermittent stretching. We have to fall back upon a comparison of the degree of tension existing in life after nerve section with the degree of atrophy found. It is, unfortunately, difficult to estimate the degree of tension which is put on the various muscles and to estimate the different effects which may be produced by prolonged slight tension, such as may occur in the flexor group of muscles, and brief violent tension which may occur in the extensor group. It is unlikely that the sitting posture of the rabbit causes anything that can be called strain on any of the muscles, for it is its normal posture, but there is some tension on the flexors. Since however the gastrocnemius and plantaris are attached to the femur, the soleus to the tibia, and the flexor dig. com. to the tibia and fibula, flexion of the thigh reduces the tension of the gastrocnemius and plantaris more than it reduces the tension of the other two muscles. In the denervated rabbit the thigh is usually flexed, but as we have seen the gastrocnemius and plantaris lose weight at a faster rate than the soleus or flexor dig. com. On the extensor side, the tibialis anticus and extensor dig. com. are both relaxed in the sitting posture, but the tibialis anticus atrophies at about the same rate as the soleus; so far indeed as the observations go, its rate is somewhat greater.

On the whole we conclude that a large factor in the rate of atrophy is the nature of the muscle fibre, and that this varies from muscle to muscle and from animal to animal. A difference in the relative number of red and white muscle fibres obviously suggests itself as the cause of the difference in rate of atrophy, but our experiments show that if this is a factor it is not the only factor.

The results given by the extensor dig. com. present an interesting problem. Whilst we think that the practically constant less loss of weight of the extensor dig. com. than of the tibialis anticus shows that its natural rate of atrophy is less, the variations found in the denervated and undenervated muscle in different experiments do not seem to be adequately explained by variations in initial weight and by a small rate of atrophy. They suggest that there was some varying condition which affected the rate. The only condition we can think of is a possible occasional greater strain, though it is difficult to see why the tibialis anticus should not be similarly affected. Theoretically this might set up inflammation which in its early stage would tend to decrease the loss of weight, and in the late stage to increase it, but in fact the muscle showed in general no sign of inflammation.

### 3. *The rate of atrophy.*

The errors involved in determining the rate of atrophy are those already mentioned (p. 52) as involved in determining the effect of treatment. One of them, viz. the wasting of the muscle on the undenervated side, will be greater. If the animals are young the error due to continued growth on the undenervated side will also be greater. A curve of the rate of atrophy can then only represent the mean of a number of observations, and the curve will be more or less different for different muscles.

A curve showing the percentage difference in weight in the gastrocnemius + plantaris + soleus after different periods of unilateral denervation in a dozen rabbits of varying age, but not fully grown, has been given by **Langley** and **Kato**(2). Control experiments to ascertain the error due to the normal difference in weight were not made on the gastrocnemius group but on the tibialis anticus + extensor dig. com. These indicated that the error was usually small, but might be about 12 p.c. The curve however was taken as showing broadly the rate of atrophy of the denervated gastrocnemius group. The general conclusions were that the muscle begins to lose weight in about three days, then loses it rapidly so that the loss at the end of the second week is about 30 p.c., and nearly 50 p.c. at the end of the fifth week, the loss being slower in the latter part of this period. The defect in this curve is that there are too few observations after the fifth day—only five out of the twelve—and none between the fourteenth and thirtieth day. All our experiments given in Table XIV were of 20 to 25 days duration and fill up a gap in the previous results. In our experiments the soleus was not taken with the gastrocnemius and plantaris; the exclusion raises the percentage difference in general by 1·5 to 1·75 p.c. If our results are plotted as those of **Langley** and **Kato** it will be found that the percentage difference in muscle weight was in one case only less than corresponds with their curve, it was greater by about 4 to 9 p.c. in six cases, and in one case by 17 p.c. Probably then the mean curve of the rate of atrophy for the muscles considered falls somewhat more steeply in the second and third week of denervation than in the curve given by **Langley** and **Kato**. The general form of the curve, at first slow, then fast, then slower and slower, appears to hold.

Some observations on the difference in weight in the leg muscles of the rabbit at varying times after unilateral section of the sciatic have been made by **Roberts**(6). He found very large irregularities in the percentage difference, and these he speaks of, as a rule, as due to variations in the rate of muscle wasting. Thus he found in one case that after 21 days denervation the tibialis anticus was 7 p.c. heavier than the corresponding muscle on the undenervated side and the soleus 28 p.c. heavier. In another case after 30 days denervation, the soleus was only 7 p.c. lighter than the undenervated muscle, whilst in a third experiment after 34 days denervation it was 69 p.c. lighter. A good many of **Roberts's** results we think can only have been due to some condition other than that of denervation. The muscles in his observations were in fact subjected before being weighed to very different degrees of exposure and stimulation, and he does not appear to have paid any regard to accessory pathological conditions, which as we have seen is a very important factor in denervation experiments. In view of his results it is perhaps not surprising that he should arrive at the conclusion that "so great is the variation in wasting to which muscle is liable that experiments in which loss of weight is taken as the standard are of little value." But it is curious that having arrived at this conclusion he should himself take the loss of weight as a standard and infer from his experiments that the tibialis anticus wastes at a slower rate than the gastrocnemius. Obviously if this is true, sound conclusions could be drawn from experiments in which this relative rate was reversed.

### *Notes on the condition of muscle during the early stage of atrophy.*

In nearly all our cases we have noted the appearance of the denervated muscles. The duration of denervation in most of these was about three weeks. Except in cases where there were marked inflammatory conditions the muscle did not look unhealthy. Even in these cases an unhealthy appearance was present only after prolonged extension, and sometimes in local patches after tendon section or ionisation. But in general the denervated muscle can be distinguished from the undenervated by its having a pinker tinge. A white muscle after denervation becomes redder, somewhat of the colour of crimson liberally mixed with white, and less transparent. The soleus becomes less lake red. The difference in tint is shown better when the animal has been bled and the muscles removed from the body. The difference is much more striking if the muscles are covered up and kept for some days. The undenervated muscles turn grey or livid, the denervated remain more or less pink till putrefaction sets in. In nearly all cases we have found that the denervated muscles kept in covered dishes liquefy earlier, and in summer grow a more plentiful crop of fungi, but we have not tested this under strict experimental conditions.

What has been said by one of us(3) about the fibrillation in the muscles of the cat holds also for the rabbit, but we have noticed occasionally a faint shimmering unaccompanied by any visible indentation in the surface of the muscle. This appears to be the feeblest form of fibrillation; it is difficult to see, on one occasion it was obvious in bright sunlight, but disappeared in diffused light at whatever angle we looked for it.

The fibrillation of some muscles presents nearly constant differences from that of some others. It has been mentioned by one of us(3) that in the cat, the fibrillation in the eight cases observed was greater in the gastrocnemius than in the soleus. In nearly all our cases in the rabbit we have noted the degree of fibrillation in the several muscles. The results, as regards the freshly exposed muscles, are as follows: with rare exceptions the fibrillation is quick in the gastrocnemius and plantaris and, judging by the extent of the indentation in the muscle fibres, it is stronger than in any of the other muscles, though it varies somewhat in different parts. In the soleus it varies greatly, in general it is quick and weak but frequently it is slight and difficult to see. The flexor dig com. and the peroneus longus have generally a quick and fairly strong fibrillation. The tibialis anticus has less obvious fibrillation except in its upper end where it is constantly distinct, the fibrillation is generally more obvious in the deep surface than in the lower part of the outer surface. It may be noticed that the sheath must be removed from the outer surface before observation. The extensor digitorum com. has as a rule less fibrillation than any other muscle, but it is also the most variable. Sometimes it is distinct and fairly strong, sometimes scattered and infrequent, sometimes quick and very weak.

It is not possible to form any accurate idea of the relative amount of chemical change accompanying different forms of fibrillation, for example when it is quick and weak in one muscle and slower but stronger in another, but from such estimate as can be formed the degree of muscle atrophy has a broad relation to the more extreme variations in fibrillation. In general, as we have seen (Table XV), the gastrocnemius and plantaris atrophy more in three weeks time than the flexor dig. com., and the flexor dig. com. more than the extensor dig. com. On the other hand, the tibialis anticus, and still more the soleus, seem often to atrophy more than corresponds to their degree of fibrillation. The experimental error attending both the degree of atrophy and the strength of fibrillation makes it impossible to compare the two with any exactness within the range of the minor differences, and, in fact, in unilateral denervation the order of percentage difference on the two sides, and in bilateral denervation the relative weights on the two sides, not uncommonly differed in one or more of the muscles from the degree of fibrillation observed at death.

We have said above that the fibrillation in the gastrocnemius and plantaris is nearly always stronger than in the other muscles. We have only found one exception. This was in a rabbit, nine days after denervation, the shortest duration of denervation in our experiments. The internal popliteal nerve had been cut on the left side. The fibrillation was very slight in the gastrocnemius and plantaris, rapid but weak in the soleus, and rapid and fairly strong in the flexor dig. com. The percentage difference in weight of the muscles on the left side, taking the right as a standard, were: gastrocnemius − 10·5, plantaris − 12·2, soleus + 0·2, flexor com. dig. − 24·6, popliteal − 23·8, tibialis anticus − 1·2, extensor dig. com. − 2·9, peronei − 0·3. So far as the gastrocnemius, the plantaris, and the flexor dig. com. are concerned the reversal of the ordinary degree of loss of weight was accompanied by a reversal of the ordinary degree of fibrillation. Incidentally the result suggests that the time of development of full fibrillation varies in different muscles, and that marked loss of weight may begin in different muscles at different times.

On the whole it appears that there is a broad correspondence between degree of fibrillation and degree of atrophy, but that there is still insufficient evidence to decide whether the fibrillation is the cause of the atrophy or merely an accompanying phenomenon.

Finally we may note that in two cases in a small area of denervated muscle there were spontaneous slow waves of contraction travelling repeatedly from a focus in both directions[1].

## General Summary and Remarks.

Our observations were made in the first place to determine the probable limits of errors in experiments on the atrophy of muscle by the bilateral denervation method, and in the second place to compare in a preliminary manner certain modifications of conditions and various methods of treatment, in the hope that some suggestions might be obtained as to the value of these in delaying the atrophy.

The normal difference in weight of the muscles on the two sides was found usually to vary within certain rather narrow limits. In 153 muscles, or small groups of muscles, of 17 animals which were weighed, 75 p.c. differed on the two sides by less than 4 p.c. and 92 p.c. by less than 6 p.c. Occasionally in some of the muscles there were greater differences, up to 9·1 p.c.

Similar comparison of weights was made after about three weeks denervation on 23 muscles of 6 animals. In these the percentage difference of weight in corresponding muscles of the two sides was greater than in the normal muscles; 43 p.c. differed by less than 4 p.c. in weight, and 73 p.c. by less than 6 p.c. The greater difference we attribute in part to slight inflammation which in two of the cases was recognisable in the leg, for in other experiments marked inflammation was found to be accompanied by more or less considerable variation in weight on the two sides, and in those in which one limb was subjected to treatment but in which there was no inflammation, the percentage differences were on the whole less than in the experiments in simple bilateral denervation. We conclude that the difference in the atrophy of corresponding muscles does not as a rule exceed 1 to 1·5 p.c. and in consequence that the data as to the difference in normal weight allow a fairly close estimate to be made of the degree of probable error in experiments on the effects of treatment by the bilateral denervation method.

---

[1] For the other conditions in which slow waves of contraction have been observed cp. Langley. This *Journal*, 50. p. 404. 1916.

The more nearly the difference in weight occurring after unilateral treatment approaches the limits which occur without treatment, the greater, obviously, is the number of experiments required to justify definite conclusions. Even when large differences are found, a reservation must be made in any individual case partly because exceptions may occur to the rule of approximate equal atrophy on the two sides, and partly because it is possible that occasionally congenital defects, or injuries in early life, may cause a considerable difference in the weight of the muscles without any other sign of their having existed.

The chief difficulty presented by experiments on the leg of the rabbit is that of preventing trophic changes in the foot and in the skin of the leg. These may cause inflammation in the leg and connective tissue growth; accompanied at first by a decreased rate of loss of weight, and later by an increased rate of loss. Post-mortem examination of muscles and bones is necessary for signs of injury that may have been incurred before operation.

In general, as regards any single experiment affecting a number of muscles, much greater weight may be attached to a negative effect of treatment than to a positive one. Normally the percentage difference in weight of the corresponding muscles on the two sides, varies in the several muscles, and any pathological condition would tend to increase the variation, consequently the chance is very slight of any treatment bringing them all within the normal limits of percentage difference. On the other hand any exceptional case, whether normal or due to pathological change, in which the weight of all the muscles was greater on the treated side would simulate the effect of a benefit by treatment.

In observing the effect of various conditions on the muscle atrophy, we have in nearly all cases allowed a denervation period of about three weeks, by the end of which time there is—with the not infrequent exception of the extensor dig. com.—a loss of weight of 30 to about 50 p.c. in the several muscles. In the majority of cases the effect was obscured by pathological change. The results obtained were as follows:

Section of the Achilles tendon caused in proportion to its nearness to the muscle connective tissue growth and an inflammatory state. The differences in weight on the two sides were greater than in simple bilateral denervation, but they were not constant in sign. In unilateral denervation experiments, the difference of weight on the two sides was at least as great when the Achilles tendon was cut, as when it was uncut. We conclude that the degree of extension of the gastrocnemius group which is caused by the sitting posture with uncut tendon has little effect on the extent of the muscle atrophy.

Continuous extension of the ankle joint for five to six hours a day caused great increase of connective tissue around the joint, and around the tibialis anticus and extensor dig. com. When the connective tissue had not seriously invaded the muscles, the muscle weight in all the extensor muscles was much greater on the extended than on the opposite side (17 to 29 p.c.). The greater weight was not due to absorption of water, and the increase of connective tissue was not sufficient to account for it.

Protracted flexion of the foot had no distinct effect.

Whilst then no appreciable error appears to arise from considerable variations of tension, the results suggest that tension beyond a certain limit, if protracted, increases in some way the final weight of the muscles.

Experiments on anæsthetised animals showed that with a sufficient galvanic current, fibrillation was stopped by ionisation with calcium chloride and less effectively by ionisation with potassium chloride. The current required for this was much too strong for use in life, but it seemed possible that daily ionisation with currents easily borne might have a cumulative action, and thus the effect observed of greatly reducing the fibrillation. In all cases however, the muscles at the time of death showed the usual degree of fibrillation.

Ionisation with calcium salt soon caused trophic changes in the skin, deposit of small lumps of calcium salt in small areas of the subcutaneous tissue and in larger areas of the superficial sheaths of the muscles; with these changes there was a growth of connective tissue. Similar but less effects were produced by ionisation with Ringer's fluid containing 0·04 p.c. calcium chloride. The galvanic current of 15–30 milliampères passed chiefly through the extensor muscles, and the changes in the skin extended but little beyond the anterior and outer surface of the leg. In three of the five experiments (Tables X, XI) the percentage difference of the flexor muscles—gastrocnemius, plantaris, soleus, flexor dig. com. and popliteus—were within, or close to, the normal limits; in one experiment (Exp. 1, Table X) there were greater variations but they were not of constant sign; finally in one experiment (Exp. 3, Table X) the flexors on the ionised side were of more or less greater weight than on the non-ionised side. The extensor group—tibialis anticus, extensor dig. com. and the peronei—though not of constant sign, were in most cases heavier on the ionised side, but it was over these muscles that the connective tissue was thickened and inflamed. In Exp. 3, Table X, the difference was very great in the tibialis anticus (42 p.c.) and in the extensor dig. com. (32 p.c.); in view however of the fact that these muscles on the non-ionised side were unusually light relatively to the body weight, it seems more likely that the great difference on the two sides was due to an abnormal condition of the non-ionised than to a beneficial effect on the ionised. In any case it seems clear that the effect on the skin makes ionisation with any solution containing calcium salt inadvisable; and that it is safer to use distilled water than tap water in making up the NaCl solution employed in passing a galvanic current through the body.

Three experiments were made on ionisation with a potassium salt. In these, there were some trophic changes, but they were slight compared with those caused by calcium salt. In two of the experiments the flexor muscles were unaffected by the ionisation; in the extensors there may have been a slight effect but this was uncertain. In the third experiment, all the muscles on the ionised side were heavier than on the non-ionised. The difference was much greater in the tibialis anticus than in any other muscle, and on the ionised side this muscle was more excitable both to single induction shocks and to the galvanic current. As we have said we think that in these experiments positive results when they occur in most of the leg muscles are less trustworthy than negative, and since no certain result was obtained in Exp. 2, conducted in much the same way, we are not inclined to lay much stress on Exp. 3 though we think it is worth repeating.

One experiment was tried with rhythmic extension and flexion, and one with massage, each for half an hour morning and evening, the massage being chiefly on the flexor group. On the whole there was a slight advantage in weight on the treated side, but as there was also slight connective tissue growth on this side, it cannot definitely be said that the treatment delayed the atrophy. It must be remembered however that there were some passive movements on the untreated side since it was not immobilised.

Experiments by the unilateral denervation method have a much larger possible error than those by the bilateral method, so that they require to be made in much larger numbers before definite conclusions can be drawn as to the effect of treatment. It is however the least unsatisfactory method of determining the rate of atrophy of different muscles, and the extent of the atrophy in a given time. In the rabbit we find that at the end of three weeks the atrophy is in nearly all cases greatest in the gastrocnemius and the plantaris, and least in the extensor dig. com. The chief factor in this difference of rate is we think the varying nature of the muscle fibres.

The extent of the atrophy of the gastrocnemius and plantaris in about three weeks was somewhat greater than is given by the curve of Langley and Kato, which was constructed from earlier and later periods of denervation, so that we think their curve falls rather too slowly in the second and third week.

The relation of the fibrillation seen at the time of death to the degree of atrophy in the rabbit is like that described by Langley(3) in the cat, *i.e.* there is a general but not a detailed correspondence. The fibrillation, as the atrophy, is nearly always greatest in the gastrocnemius and plantaris, and commonly weakest in the extensor dig. com.; on the other hand the fibrillation in the soleus was usually less than corresponded with its atrophy.

Denervated white muscle takes on a pinker tinge; denervated red muscle becomes less dark red.

Reviewing the effect of the various forms of treatment we have tried there is we think only one which gives any hope of considerably reducing the rate of atrophy, viz. ionisation with a potassium salt, and that is slender, for an (apparently) positive result was only obtained in one out of three experiments.

The general impression we get from our experiments and from those made earlier by one of us, is that none of the methods of treatment of denervated muscle now in use—passage of galvanic current, production of contraction, passive movements and massage—can have more than a slight effect in delaying muscle atrophy and that any beneficial effect they may have could be produced with much less expenditure of time and money than is now spent on them. The conditions in man allow of very little exact observation. The time of recovery, some 6 to 10 months, varies so greatly with the unascertainable conditions at the point of suture that any influence there may be of other treatment is completely dwarfed. Connective tissue growth may easily be caused, and on that account measurements of the limbs at intervals of stimulation is a very insecure basis for estimating growth of muscle; moreover if there were a growth of the muscle it would mean that the atrophy could be prevented altogether, and no one now, so far as we know, claims that this can be done. The only remaining method in man is to compare the electrical irritability after the muscle has been left alone with that after it has been treated, a method by no means valueless, but in which the experimental error is very large.

|| NB

The sole way at arriving at exact results as to the effect of the several forms of treatment on muscle atrophy is by experiment on animals. It is merely a question of making a sufficient number of experiments, keeping a careful eye on the various possibilities of experimental error. The form of experiment we have chosen strictly speaking only applies to the effect of treatment during the early stage of atrophy. But if treatment has no effect during the period when muscle dwindles to about half its size, it hardly seems likely that it will delay the later dwindling. The question cannot however be definitely settled on this ground, for the conditions are different after some four or five weeks have elapsed; the arteries gradually recover tone, and possibly the daily quantity of blood flowing through the muscles falls below normal.

Finally to avoid misconception we would point out that in what we have said we are not dealing with the effect of treatment when the nerve has begun to make functional connexion with the muscle, but solely with its effect on the denervated muscle.

## REFERENCES.

(1) **Langley.** Journ. of Physiol. 50. p. 337. 1916.
(2) **Langley and Kato.** Ibid. 49. p. 432. 1915.
(3) **Langley.** Ibid. 51. p. 377. 1917.
(4) **Reid.** Edinburgh Monthly Journ. Med. Science, May, 1841 (quoted from reprint in Physiol. Anat. and Path. Researches, Edin. 1848).
(5) **Brown-Séquard.** C. R. des Séances et Mém. d. l. Soc. de Biol. (Paris), 1849, p. 195, Dec. See also Journ. d. l. Physiol. 2. p. 75 1859.
(6) **Roberts.** Brain, 39. p. 297. 1916.

# ON THE CREATIN CONTENT OF SKELETAL MUSCLE IN DEGENERATION FOLLOWING DENERVATION.

By E. P. CATHCART, P. S. HENDERSON and D. NOËL PATON.

*(From the Physiological Laboratory, University of Glasgow.)*

RECENT work on the physiology of skeletal muscle indicates that there are two elements in contraction—a rapid sharp response to stimulation, and a more sustained contraction of the nature of a tonus which plays an important part in such prolonged actions as are involved in the maintenance of posture. The dual nature of the contraction is indicated by evidence of different kinds.

1. It has been for long known that there is a difference in the contraction of white and red muscles—the former contracting rapidly, the latter more slowly. The fact that in the latter the sarcoplasm is more abundant and that in the former the sarcostyles constitute the greater part of the sarcous substance seems to indicate that the sarcostyles have developed as the elements concerned in the quick response, while the sarcoplasm is the part to which the slower, sustained action is due. The differentiation of the skeletal muscles from the visceral types is due to the development of those sarcostyles. The theory of the functional difference of sarcostyles and sarcoplasm was put forward by Bottazzi(1), who has applied it to a large number of the phenomena of the contraction of muscles, including the peculiar reaction to such drugs as veratrin.

2. Roaf(2) observed that in decerebrate rigidity the gaseous metabolism—*i.e.* output of $CO_2$ and intake of $O_2$ is not increased as it is in ordinary contractions.

3. De Boer(3) describes and figures a double innervation of skeletal muscle, and the experimental work of Boeke(4) and of Kure, Hiramatsu and Naito(5) seems to support the view that while the ordinary cerebrospinal fibres produce rapid contraction, the sympathetic fibres may be concerned in the tonic contraction. Although the more recent investigations of Rijnberk(6) do not confirm all these results, they lend support to the conception of a double element in muscular contraction.

4. In denervated muscle a series of changes occur which are generally described as the result of degeneration. First the response to rapid induction shocks is decreased and gradually lost, while the response to the slower galvanic make and break shocks is increased, and the contractions become more sluggish and more sustained. Tracings showing the course of the change in single muscles have been given by **Roberts**(7). By the end of the third week, when the sluggish character is very marked, the cross stripings have been described by a number of observers as being less distinct.

**Pekelharing** and his co-workers have associated the condition of tonus with the presence of creatin in muscle, in spite of the fact that their analyses show (8) that creatin is much more abundant in white than in red muscle. In white muscle their average as creatinin is 4·56, in red 2·92 mg. per grm., and the results of **Cabella**(9) are in the same direction. They base their conclusions on their observations: (1) that conditions which increased the tone of muscle increase its creatin content; (2) that section of the posterior spinal roots which decreases the tone decreases the creatin content(10), and (3) that increased tonus is associated with an increased output of creatinin in the urine. (See also **Spriggs**(11) and **Shaffer**(12).) This somewhat begs the question of the relationship of urinary creatinin to muscle creatin. These results have not always been confirmed by other workers. **Weber**(13) found a decrease in creatin content in dog's muscle after cinchonine convulsions, which increase the tonus. Nor does their conclusion seem to be supported by the very small amount of creatin in visceral muscle(14) where the element of tonus in the contraction is so prominent.

In the course of an investigation on tetania parathyreopriva, one of us (D. N. P.) had occasion to cut the sciatic nerve in a series of animals, and it seemed of interest to investigate whether there is any change in the creatin content of the denervated muscles as they undergo the process of degeneration.

On the subject we have been able to find two previous observations. In 1908 **Weber**(13) found in one dog that the creatin content of muscle was decreased 39 p.c. in the muscle several weeks after section of the sciatic nerve. **Pekelharing** and **Van Hoogenhuyze**(10) cut the sciatic nerve on one side in three rabbits, and killed the animal three days later. They found a slight loss in weight in the paralysed muscles, as compared with the normal, and a loss of creatin of 11·3 p.c.

The nerve sections were made by D. N. P. and **Alex. Watson**. The electrical reactions were taken in some animals by D. N. P. in the others by P. S. H. The creatin analyses of cats I, II, IV, VI, VIII and XII, and on rabbits I and II were made by E. P. C., those in the others by P. S. H. The method used was that described by Brown and Cathcart(14) depending upon Folin's colorimetric method. Since the personal equation modifies the readings the results of each observer are comparable with one another, but not with those of the other. The results are expressed as creatinin.

Twelve cats were used and two rabbits. The sciatic nerve of the left hind leg was severed high up in the thigh, a piece of the nerve being removed to prevent regeneration. The cats were confined in a room in the laboratory, but had plenty of space in which to move about. The wounds healed directly. The left hind leg was limp, but the muscles above the knee supplied by the anterior crural, could be moved and the leg was not dragged.

In most of the animals the electric excitability of the muscles was employed as an index of the onset of degeneration. The reactions were taken at intervals varying from 2 to 4 days, and the evidence of degeneration was usually observed during the second week, from 9 to 13 days after the operation. This corresponds with the results of Noël Paton and Findlay(15) and with those of Roberts(7).

In five of the cats parathyroidectomy was performed, in the others nerve section alone. The animals were killed, from the third to the twenty sixth day, and in the case of one rabbit the fifty second day after section of the nerve. In each case the gastrocnemii of both hind legs, were excised and rapidly weighed, after removal of all traces of fatty tissue, and the creatin content estimated as stated.

In a preliminary histological examination (by P. S. H.) it was found that the soleus muscle after 22 days denervation had indistinct longitudinal and cross striation and the nuclei appeared to be increased. The average diameter of the fibres was ·012 mm. against ·024 mm. on the normal side.

The following table gives the results.

| No. of Exp. | Nature of operation | Period after section (in days) | E.R.—KOC (i) on day of operation (ii) on day of death | Weight of muscle | | Percentage creatin content | | Percentage difference in creatin | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | N. | D. | N. | D. | Rise | Fall | |
| I | N.S. + P. | 3 | 1·5<br>0·4 | 28·1 | 27·1 | ·26 | ·27 | 4·0 | — | Both operations on same day. Bread and milk diet. |
| II | N.S. + P. | 3 | 2·0*<br>5·0 | 24·9 | 23·6 | ·31 | ·35 | 12·9 | — | Both operations on same day. Beef diet.<br>* Whole leg moved, difficult to determine. |
| III | N.S. + P. | 7 | 3·5<br>4·0 | — | — | ·25 | ·21 | — | 16·0 | Parathyroids removed 4 days after N.S. |
| IV | N.S. | 11 | 6·0<br>14·0 | 9·35 | 8·9 | ·261 | ·281 | 7·66 | — | Died under chloroform while reactions were being taken. |
| V | N.S. | 15 | 4·0<br>0·9 | 10·7 | 8·2 | ·297 | ·25 | — | 15·8 | |
| VI | N.S. | 15 | — | 24·55 | 15·25 | ·24 | ·19 | — | 20·8 | |
| VII | N.S. | 16 | 6·0<br>1·0 | 4·4 | 2·7 | ·31 | ·266 | — | 14·2 | |
| VIII | N.S. + P. | 22 | 5·5<br>4·0 | 8·05 | 7·12 | ·31 | ·24 | — | 22·5 | Parathyroidectomy 19 days after N.S., after onset of degeneration. |
| IX | N.S. | 22 | 8·0<br>6·0 | 8·3 | 6·2 | ·29 | ·20 | — | 31·0 | |
| X | N.S. | 24 | 7·0<br>0·6 | 11·14 | 9·28 | ·285 | ·18 | — | 36·8 | |
| XI | N.S. | 26 | 6·0<br>1·0 | 6·7 | 5·1 | ·319 | ·218 | — | 31·6 | |
| XII | N.S. + P. | 26 | 3·4<br>1·7 | 7·7 | 4·0 | ·25 | ·09 | — | 64·0 | Parathyroidectomy 23 days after N.S., increase of E.R. not manifest till after 2nd operation. |
| Rabbits | | | | | | | | | | |
| I | N.S. | 12 | — | 10·67 | 8·81 | ·42 | ·38 | — | 9·7 | |
| II | N.S. | 52 | 10·0<br>0·7 | 9·36 | 4·99 | ·25 | ·12 | — | 52·0 | |

N.S. nerve section; P. parathyroidectomy; N. normal; D. degenerate; E.R. electric reactions; KOC kathodal opening contraction.

## CONCLUSIONS.

Up to the 11th day there seems to be no distinctive change in the creatin content of the muscle. But after the 15th day, when the reaction of degeneration is well established there is a steady and progressive decrease in the creatin content, which becomes very pronounced between the third and fourth week.

The result of these observations do not lend support to the theory of the special relationship of creatin to the sarcoplasmic element of skeletal muscle but taken in conjunction with the greater amount of creatin found by Pekelharing and by Cabella in white muscle, and with the very small creatin content of visceral muscle, would rather tend to point to the association of creatin with the sarcostyle element of the fibre.

The expenses of this investigation were defrayed by the Medical Research Committee.

## REFERENCES.

(1) Bottazzi. Journ. of Physiol. 21. 1. 1897.
(2) Roaf. Quart. Jl. Exp. Physiol. 5. 31. 1912.
(3) De Boer. Zs. Biol. 65. 239. 1915.
(4) Boeke. Anat. Anz. 44. 343. 1913.
(5) Kure, Hiramatsu and Naito. Zbl. Physiol. 28. 130. 1914.
(6) Van Rijnberk. Arch. Néerland. de Physiol. 2. 4. 1917.
(7) Roberts. Brain, 39. 1916.
(8) Pekelharing and van Hoogenhuyze. Zs. f. physiol. Chem. 64, 266. 1910.
(9) Cabella. Zs. f. physiol. Chem. 84. 29. 1913.
(10) Pekelharing and van Hoogenhuyze. Kon. Akad. v. Wetensch te Amsterdam, 18. 1916.
(11) Spriggs. Quart. Journ. Med. 1. 63. 1907–8.
(12) Shaffer. Amer. Journ. of Physiol. 23. 1. 1908–9.
(13) Weber. Arch. f. exp. Path. 58. 93. 1908.
(14) Brown and Cathcart. Biochem. Journ. 4. 420. 1909.
(15) Noël Paton and Findlay. Journ. of Physiol. 50. 193. 1916.

# ON THE POSSIBILITY OF MIXING THE AIR IN THE LUNGS WITH FOREIGN AIR, ESPECIALLY AS IT IS USED IN KROGH AND LINDHARD'S NITROUS OXIDE METHOD. BY CARL SONNE, M.D.

IN a recent number of this journal(1) Krogh and Lindhard gave experiments in support of their contention that in their method for determining the blood circulation rate, they obtain a sufficiently uniform mixture of alveolar air and the nitrous oxide administered. In previous papers(2) I have maintained that this is not so. I have made further experiments on this question; the results, as my former ones, show that Krogh and Lindhard's method for circulation rate determination is unserviceable. In giving these results I take the opportunity of offering some other criticisms on Krogh and Lindhard's method.

I may first take the experiments of Krogh and Lindhard which appear most in their favour, viz. those on p. 87 in which they determine the volume of oxygen absorbed per litre blood, and where they find that the volume of oxygen taken up by the same individual is to a certain extent independent of the number of mixing respirations applied in the introductory period. As they correctly observe, "if the pulmonary gases are incompletely mixed by three respirations we must expect that by using more mixing respirations of equal depth and rate in the introductory period, we should obtain a higher figure for the oxygen taken up per litre blood." With regard to these experiments it may be observed: (1) that the depth and rate of the mixing respirations in the introductory period were not measured, and this, as will be obvious from the following, is essential; and (2) that in the case of two of the subjects at any rate a considerably increased absorption of oxygen (up to 40 %) was actually found when five respirations were mixed.

But the latter result is, according to Krogh and Lindhard, "of course simply due to the fact that a prolonged deep respiration will affect the circulation itself." That this is the case they do not however show, so that it is simply an hypothesis.

I have myself made a series of similar experiments.

TABLE I.

| | | The mixing respirations | | | Sample I | | | Sample II | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | No. | No. | Average depth | Frequency | $CO_2$ | $O_2$ | $N_2O$ | $CO_2$ | $O_2$ | $N_2O$ | Oxygen absorption per litre blood |
| C. L. | 1 | 3 | 1·6 | 14 | 4·64 | 17·47 | 9·92 | 5·86 | 15·35 | 7·88 | 42 |
| (vital capacity | 2 | 4 | 1·7 | 11 | 5·19 | 15·55 | 9·72 | 6·11 | 13·66 | 8·36 | 53 |
| 3·7 litres) | 3 | 4 | 2·4 | 11 | 5·87 | 14·47 | 9·74 | 5·92 | 11·71 | 8·47 | 72 |
| | 4 | 4 | 2·4 | 11 | 3·97 | 17·57 | 11·53 | 5·92 | 14·81 | 10·13 | 81 |
| | 5 | 5 | 2·5 | 14 | 5·28 | 16·61 | 11·79 | 5·47 | 15·01 | 11·08 | 92 |
| A. T. | 6 | 3 | 1·6 | 14 | 3·91 | 16·84 | 11·99 | 5·55 | 14·58 | 9·94 | 50 |
| (vital capacity | 7 | 3 | 2·1 | 12 | 3·69 | 17·54 | 14·35 | 5·37 | 15·84 | 12·63 | 57 |
| 3·0 litres) | 8 | 4 | 2·1 | 13 | 4·21 | 17·91 | 12·71 | 5·40 | 16·09 | 11·23 | 60 |
| | 9 | 3 | 2·1 | 14 | 3·66 | 18·63 | 12·03 | 5·39 | 16·25 | 10·42 | 67 |
| | 10 | 5 | 2·0 | 13 | 4·34 | 18·45 | 14·59 | 5·83 | 16·56 | 13·15 | 72 |
| | 11 | 5 | 2·0 | 14 | 3·79 | 18·31 | 13·82 | 5·62 | 16·46 | 12·55 | 75 |
| | 12 | 5 | 2·1 | 13 | 4·58 | 17·60 | 14·77 | 5·84 | 15·72 | 13·59 | 91 |
| A. N. | 13 | 3 | 2·2 | 18 | 3·81 | 16·96 | 12·64 | 5·91 | 14·28 | 10·52 | 61 |
| (vital capacity | 14 | 3 | 3·0 | 19 | 3·60 | 17·91 | 14·42 | 5·40 | 15·76 | 12·72 | 70 |
| 3·7 litres) | 15 | 5 | 2·5 | 21 | 4·27 | 18·08 | 12·81 | 5·75 | 15·88 | 11·56 | 84 |
| | 16 | 4 | 3·2 | 19 | 4·15 | 18·51 | 13·00 | 6·02 | 15·49 | 11·46 | 92 |

In the table I have given not only the number of the mixing respirations, but also their exactly measured and calculated average depth and their frequency (calculated per minute). The determinations are arranged in a consecutive order so that in the case of each subject the results showing the lowest value of oxygen taken up are placed at the top, then come the next lowest and so on. It cannot of course be assumed that the volume of the lungs with which the mixing respirations are begun is quite identical in all the experiments, nor that the lungs have distended and contracted quite uniformly, but in spite of this, it will easily be seen that we here have an absolute tendency towards a higher value of oxygen taken up when we increase the number and depth of the mixing respirations. The effect derived from variation of frequency is not clearly seen from these experiments where the frequency for each individual is approximately the same, but what is obvious is the significance of an even proportionally slight variation in the depth of the respirations, so that we may find a larger amount of oxygen absorbed by four than by five mixing respirations, if only the depth has been sufficiently larger by the four respirations. (Determinations 15 and 16.) The value of such experiments is thus very problematic if the depth and frequency of the mixing respirations have not been exactly measured and stated.

But to what may we ascribe the systematic variation in the results appearing on systematic variation of the mixing respirations in the introductory period? It has not so far been proved in this paper that this is due to an incomplete mixing of the pulmonary gases, that is to a variation of the degree of mixing. For it might be possible that an increase of the intensity of the mixing respirations could effect a change in the circulation in a systematic way, and if so, this might already be visible with four mixing respirations, and not only with five or six as assumed by Krogh and Lindhard.

The first thing we must investigate is: what may reasonably be required as to the uniformity of mixing of the pulmonary gases according to Krogh and Lindhard's method? What samples of air are here taken for analysis? When the pulmonary gases at a specified time—that is at the end of the introductory period—are considered to be sufficiently mixed, so much air is expired (800–1000 c.c.) that the dead space becomes thoroughly washed out, and a sample of the last expired alveolar air furnishes the first sample for analysis; the breath having then been held a certain time, a rapid expiration follows, as deep as possible (to the residual air in the lungs), after which the now last expired alveolar air furnishes the second sample for analysis. The condition that the results thus obtained may be efficient for the calculation of the blood circulation rate, is, that the variation found by analysis of the two air samples are exclusively contingent upon the exchange of the blood gases and the pulmonary gases, or, in other words, that if the period in which the breath is held is reduced to a minimum,—that is practically equal to 0—there must not be a larger difference between the two samples of air than one corresponding to the exchange during the short time of expiration between the two drawings of samples. It is the air of two such samples which it is of interest to get examined in this connection.

I obtained the two samples of air by expiring through a three-way tap,—like the one used by Krogh and Lindhard (fig. 1, p. 65),—first 800–1000 c.c. through a side tube into a spirometer, and then, after a quick turn of the tap, which practically takes no time and which is not felt by the subject as an interruption of the expiration; the expiration is continued, as deep as possible, into another spirometer through the other side tube connected with the tap. From two sample-taking tubes, one in each of the side tubes connected by the tap, I then drew the two samples for analysis.

In this way I have examined numerous mixtures of pulmonary gases according to the mixing respirations of the introductory period, and I have constantly found the same results, so I shall only here state some of the series of experiments.

TABLE II.

| No. | Subject | Entered into the lungs before the mixing respirations into spirometer I | Mixture of gases in spirometer I into which the mixing respirations were made | The average depth and frequency of the three mixing respirations | | The time of expiration between the taking of the first and second sample in seconds | Sample I | | | Sample II | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Depth in litres | Frequency | | $CO_2$ | $O_2$ | $N_2O$ | $CO_2$ | $O_2$ | $N_2O$ | |
| 1 | A. T. | Atmospheric air | $N_2O$ mixture as in usual circulation experiments | 1·9 | 13 | 1·5 | 4·66 | 14·33 | 13·27 | 5·19 | 13·88 | 12·34 | *Too large decrease* of $N_2O$ % during expiration |
| 2 | do. | do. | do. | 1·5 | 13 | 1·8 | 4·23 | 14·23 | 13·11 | 5·23 | 13·88 | 11·91 | do. |
| 3 | do. | do. | do. | 1·7 | 13 | 1·8 | 4·52 | 14·27 | 15·49 | 5·05 | 13·77 | 14·28 | do. |
| 4 | A. N. | do. | do. | 1·6 | 20 | 1·5 | 4·34 | 14·89 | 13·52 | 4·99 | 14·45 | 12·24 | do. |
| 5 | A. T. | High $O_2$ mixture from spirometer II | do. | 2·0 | 13 | 1·5 | 4·08 | 23·55 | 18·41 | 4·79 | 24·15 | 16·62 | Same, *increase* of $O_2$% during expiration |
| 6 | do. | do. | do. | 2·2 | 12 | 1·5 | 3·90 | 26·17 | 16·48 | 4·29 | 26·86 | 15·28 | do. |
| 7 | A. N. | do. | do. | 2·2 | 17 | 1·5 | 4·41 | 30·27 | 16·15 | 5·05 | 31·18 | 14·88 | do. |
| 8 | do. | $N_2O$ mixture from spirometer II | Atmospheric air + plenty of $O_2$ | 2·6 | 16 | 2·1 | 4·24 | 44·20 | 7·66 | 4·98 | 42·22 | 8·07 | Too large *decrease* of $O_2$ % and *increase* of $N_2O$ % during expiration |
| 9 | do. | $N_2O$ and $O_2$ mixture from spirometer II | Atmospheric air | 2·4 | 15 | 1·8 | 4·39 | 23·42 | 8·04 | 5·04 | 23·29 | 8·77 | *Increase* of $N_2O$ % during expiration |
| 10 | A. T. | do. | Atm. air + ordinary expiration air | 1·9 | 12 | 1·6 | 4·35 | 23·50 | 7·18 | 4·67 | 23·82 | 7·80 | *Increase* of $O_2$ % and $N_2O$ % during expiration |
| 11 | A. N. | do. | do. | 2·6 | 16 | 1·5 | 5·94 | 20·65 | 6·26 | 6·11 | 20·72 | 6·50 | do. |

In the experiments given in Table II the mixing respirations and the drawing of air samples were made exactly as in Krogh and Lindhard's equilibrium method, but the pause in respiration was reduced practically to 0. The first four experiments were made with $N_2O$ mixtures, such as are usually applied, and the mixing respirations were in immediate continuation of the natural respiration in atmospheric air; corresponding to the computed, rather long time for expiration which will always elapse before the subject has expired to the maximum depth, we find a rather considerable increase in the $CO_2$ percentage and decrease in the $O_2$ percentage. The decrease in the $N_2O$ percentage, which is also bound to occur, will be seen to be appreciably larger than might be expected. It is however impossible accurately to predict the size of this decrease, so that the first four determinations in the table go scarcely further than to arouse a suspicion that the mixing has not been sufficiently uniform. The case is however different with the following experiments in the table. Immediately preceding the three mixing respirations, I have introduced a foreign mixture of gases into the lungs (column 3 of the table) by two to three very deep respirations into the second spirometer with this air (spirometer II), after which the tap was turned into connection with the proper mixing spirometer (spirometer I), into which the prescribed three mixing respirations were made with the gas mixture stated in the table (column 4), and then samples were taken as previously described. If the mixing after the three respirations had been complete —as maintained by Krogh and Lindhard—an analysis of the two samples should constantly appear with a decrease of the oxygen and nitrous oxide percentages corresponding to the time of expiration. It will however be seen that this is far from being the case. In exps. 5, 6, 7, 10 and 11, in which a high oxygen mixture has been taken into the lungs before the three mixing respirations, there appears a considerable increase in the oxygen percentages during expiration, and in exps. 8, 9, 10 and 11, in which a nitrous oxide mixture has been taken into the lungs before the mixing respirations performed with the air mixture free from $N_2O$ in spirometer I, there appears a considerable increase in the nitrous oxide percentages during expiration. It is possible, then, at pleasure, systematically to procure a fall or rise in one or both percentages, and in consequence the experiments prove that the alveolar air is not uniformly mixed after the three mixing respirations prescribed in Krogh and Lindhard's equilibrium method.

What influence may the error caused by incomplete mixing have on the circulation rate determination according to Krogh and Lindhard's method? Is it so large as to be essential with regard to the efficiency of the method? In other words, can it be proved that the varying results in Table I are exclusively due to the varying degree of incomplete mixing of the gases? Although the results of the experiments in Table II go far to show that this is so, it is not possible from these results to state accurately the size of the error, because the absorption of nitrous oxide by the blood though rather considerable, cannot be stated exactly. To get a more exact figure for the error, I applied another gas in the experiments given in Table III, viz. hydrogen, which is only to a very slight extent absorbed by the blood. The experiments were, otherwise, made in the same way as in Table II, except that. Table III also gives some experiments with four or five mixing respirations. The depth and frequency of the mixing respirations were also measured. Immediately preceding the mixing respirations, hydrogen was introduced into the alveolar air by two or three extremely deep respirations into spirometer II, which contained a high hydrogen mixture, and then the mixing respirations were made into spirometer I, which contained ordinary atmospheric air. If the gases were then completely mixed, the hydrogen percentages of the two alveolar air samples should be almost identical, owing to the fact that the variation of capacity can only be very small on account of the usually slightly larger absorption of oxygen than exchange of carbon dioxide during the comparatively short time of expiration. As might be expected from the experiments in Table II, the hydrogen percentage was however constantly higher in the second than

TABLE III.

| | | The mixing respirations | | | Hydrogen percentage of | | | |
|---|---|---|---|---|---|---|---|---|
| Subject | No. | Number | Average depth | Frequency | Sample I | Sample II | Difference | Difference in percentages of Sample I |
| A. T. | 1 | 3 | 1·6 | 14 | 11·50 | 13·22 | 1·72 | 15·0 |
| | 2 | 3 | 1·4 | 14 | 14·44 | 16·04 | 1·60 | 11·1 |
| | 3 | 3 | 2·0 | 15 | 6·40 | 6·72 | 0·32 | 5·0 |
| | 4 | 5 | 2·3 | 17 | 7·50 | 7·84 | 0·34 | 4·5 |
| | 5 | 5 | 1·8 | 16 | 7·80 | 8·14 | 0·34 | 4·4 |
| | 6 | 5 | 2·2 | 16 | 8·92 | 9·28 | 0·36 | 4·0 |
| | 7 | 5 | 1·9 | 15 | 7·92 | 8·14 | 0·22 | 2·0 |
| A. N. | 8 | 3 | 2·5 | 14 | 9·18 | 10·04 | 0·86 | 9·4 |
| | 9 | 3 | 2·0 | 10 | 12·44 | 13·40 | 0·96 | 7·7 |
| | 10 | 4 | 1·3 | 9 | 15·66 | 16·80 | 1·14 | 6·6 |
| | 11 | 4 | 2·4 | 22 | 7·34 | 7·74 | 0·40 | 5·4 |
| | 12 | 5 | 2·9 | 20 | 6·00 | 6·20 | 0·20 | 3·3 |
| | 13 | 5 | 3·0 | 20 | 13·36 | 13·64 | 0·28 | 1·4 |
| | 14 | 3 | 2·5 | 28 | 7·86 | 7·96 | 0·10 | 1·3 |

in the first sample, as a consequence of the incomplete mixing. Not even with five mixing respirations was the mixture complete.

The experiments are arranged according to the same principle as in Table I, so that, in the case of each subject, the experiments showing the highest difference (that is the difference of percentage measured in the hydrogen percentage of sample I) are placed at the top, then follow the next highest and so on (last column). It will be seen that the better the mixing, the smaller is the difference. The effect derived from variation of frequency appears for instance in exps. 8 and 14; in the latter, all other conditions being equal, the rate of respiration was doubled and the difference consequently much diminished. On comparing the experiments in this table with those in Table I, we find very great congruity: the completer the mixing, the smaller is the difference between the hydrogen percentages, and the higher the absorption of oxygen found, and *vice versa*. The extent of this congruity will be further emphasized by the following considerations and calculations.

If, as is to a certain extent possible, a steady rise in the hydrogen percentage occurs in the course of the last deep respiration, and we suppose there was exactly double the volume of air in the lungs when the first sample was drawn as when the second was drawn, the hydrogen percentage of sample II should exactly represent the average percentage of the air volume in the lungs at the moment of the first drawing of samples, and the difference between the two hydrogen percentages should thus exactly represent the incompleteness of mixing of the alveolar air at the time stated. In the experiments given it is however likely that the volume of air expired between the first and second taking of samples may exceed the volume left as residual air in the lungs after the second sample is taken, so that the mentioned average percentage, if anything, will be a little lower than the hydrogen percentage in sample II, and the difference between the hydrogen percentage of sample I and the average percentage should thus probably be slightly lower than the stated difference of percentages between sample I and II.

To get an idea of the effect of the incomplete mixing on the results of the circulation determinations in Table I, it is necessary, by means of the figures in Table III, to determine as accurately as possible the extent to which the nitrous oxide percentage[1] of sample I exceeds the average nitrous oxide percentage of the total alveolar air at the moment when the first sample is taken, assuming the air to be sufficiently mixed when the second sample is taken after the breath has been held for a few seconds, so that the $N_2O$ percentages of sample II need no correction. The following will serve as an example of the calculation. In exp. 13 in Table I, three respirations of an average depth of 2·2 litres and a frequency of 18 were mixed. Exp. 8 in Table III is the one which comes nearest in mixing, it was only slightly deeper but on the other hand not quite so rapid; this experiment shows a difference between the hydrogen percentages of 9·4 %; if now exp. 13 in Table I had been a hydrogen experiment (as in Table III), we should expect to find a difference of about 9 % between the hydrogen percentages. This signifies, in so far as circulation determination is concerned, that the nitrous oxide percentage of sample I is about 9 % too high. How much 9 % exceeds the actual value we cannot, as previously said, predict exactly; but if we consider the two experiments 13 and 15 in Table I, where the degree of mixing was varying in strength, we find, on reducing the $N_2O$ percentage by $a \times 9$ % in the first case and by $a \times 3$ % in the second case, that the absorption of oxygen becomes identical (93 and 104) when $a$ is equal to 0·8. On recalculating in this way all the determinations in Table I, placing 0·8 everywhere for $a$, we obtain the following results.

---

[1] On applying oxygen percentages approximately identical to the naturally existing alveolar oxygen tension, there cannot be any important corrections to make.

TABLE IV.

(Table I with recalculated oxygen absorption, the mixing error being eliminated as far as possible.)

| | | The mixing respirations | | | The $N_2O$ % found in sample I | | $N_2O$ % after reduction | Oxygen absorption | |
|---|---|---|---|---|---|---|---|---|---|
| Subject | No. | Number | Average depth | Frequency | | $N_2O$ % reduced by | | found | after reduction |
| A. T. | 6 | 3 | 1·6 | 14 | 11·99 | 0·8 . 12·0 = 9·6 % | 10·84 | 50 | 97 |
| | 7 | 3 | 2·1 | 12 | 14·35 | 0·8 . 6·0 = 4·8 % | 13·66 | 57 | 86 |
| | 8 | 4 | 2·1 | 13 | 12·71 | 0·8 . 5·0 = 4·0 % | 12·20 | 60 | 83 |
| | 9 | 3 | 2·1 | 14 | 12·03 | 0·8 . 5·5 = 4·4 % | 11·50 | 67 | 91 |
| | 10 | 5 | 2·0 | 13 | 14·59 | 0·8 . 3·0 = 2·4 % | 14·24 | 72 | 91 |
| | 11 | 5 | 2·0 | 14 | 13·82 | 0·8 . 3·0 = 2·4 % | 13·49 | 75 | 99 |
| | 12 | 5 | 2·1 | 13 | 14·77 | 0·8 . 3·0 = 2·4 % | 14·42 | 91 | 112 |
| A. N. | 13 | 3 | 2·2 | 18 | 12·64 | 0·8 . 9·0 = 7·2 % | 11·73 | 61 | 93 |
| | 14 | 3 | 3·0 | 19 | 14·42 | 0·8 . 5·0 = 4·0 % | 13·84 | 70 | 99 |
| | 15 | 5 | 2·5 | 21 | 12·81 | 0·8 . 3·0 = 2·4 % | 12·50 | 87 | 104 |
| | 16 | 4 | 3·2 | 19 | 13·00 | 0·8 . 2·0 = 1·6 % | 12·79 | 92 | 102 |

In the last column I have given the oxygen absorption found through this recalculation, with the striking result that this has become identical for all the determinations. By eliminating, as accurately as at all possible, the error caused by incomplete mixing found in the experiments in Table I, it thus appears that we arrive at identical results. There is thus no ground to suppose that the variations in the results of the determinations in Table I are due to anything but the different degree of incomplete mixing, and Krogh and Lindhard's contention that an increased mixing respiration in the introductory period may cause a rise in the absorption of oxygen "simply because the circulation itself has been affected" is certainly not tenable, at least not to the extent held by them.

I may now pass to the other experiments made by Krogh and Lindhard (1. pp. 85, 86) to demonstrate that the alveolar air has been uniformly mixed by the three mixing respirations prescribed according to the equilibrium method. These experiments were performed in the following manner: after the prescribed mixing respirations into a spirometer through a three-way tap with a mouth-piece, the tap is turned, and a deep expiration is made into a second spirometer, after which a sample of the last expired alveolar air is taken for analysis. The air in the second spirometer having been mixed by means of a revolving fan, a sample of this air is also taken for analysis. From the analyses, the volume of the expiration, the initial volume of air in the expiration spirometer and the dead spaces in the air passages, the tap and the tubing, the composition of the expired alveolar air is calculated. This ought then to correspond to that directly found, which is however naturally somewhat more "utilized." In these experiments the mixing was performed both according to the residual method and according to the equilibrium method. In this paper I have exclusively applied the equilibrium method, since this has been most universally used, and since the error caused by incomplete mixing must be approximately the same for both methods. The following comments are therefore equally applicable to all Krogh and Lindhard's experiments.

1. Several of Krogh and Lindhard's results show that there are flaws in the efficiency of their method for examining the uniformity of the alveolar air. When, for instance, they have mixed with a nitrous oxide mixture in the first spirometer, the $N_2O$ percentage found in the last expired alveolar air should of course be lower than the calculated average percentage of the expired alveolar air. In the first experiments in Table 15 (p. 86) the $N_2O$ percentage found is however higher than the one calculated, and that even to a very considerable extent. It would be natural to suppose that identical relations would be found with regard to carbon dioxide, so that the $CO_2$ percentages found would prove to be slightly higher than those calculated. As will be seen in Tables 14 and 15 Krogh and Lindhard have however come to the opposite result in some of their experiments.

I have myself found, on a previous occasion (2), that by producing a forced deep, maximum expiration a slightly higher value for $CO_2$ % may occasionally be found in the last expired alveolar air; but in the present case nothing is said about the expiration having been maximum.

2. If the differences between the found and the calculated percentages always corresponded exactly to the time of expiration, the method for examining the uniformity applied by **Krogh** and **Lindhard** might perhaps be satisfactory; but, in the first place, the volume of the gas exchange during the time of expiration cannot be exactly predicted, at any rate not so far as the nitrous oxide is concerned, and, secondly, **Krogh** and **Lindhard** admit that, if anything, their experiments tend to show that the mixture of gases has not been absolutely complete, only not so incomplete as to play any part in the results of the circulation rate determinations. The fact is, that it is not at all the average alveolar air percentage in a "deep" expiration sample that is of main importance.

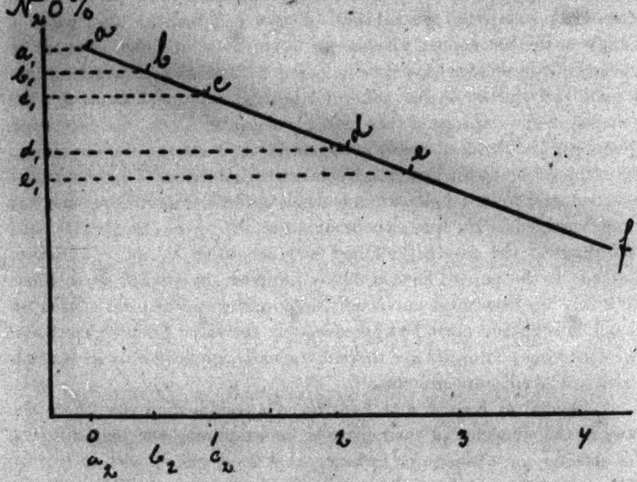


Fig. 1.

What is really essential will be most distinctly seen from this curve in which the line *abcdef* represents the fall in the $N_2O$ percentage of the alveolar air during an expiration to complete evacuation of the lungs, which I suppose may be fairly well represented to occur according to a right line; $e$ stands for the $N_2O$ percentage at the deepest possible expiration. What it is now of importance to ascertain with regard to the circulation rate determination is, whether the difference between $a_1$ (*i.e.* the percentage of the first expired alveolar air) and $d_1$ (*i.e.* the average percentage of the total alveolar air) is so large that this error may affect the results appreciably. But this is something quite different from what **Krogh** and **Lindhard** examine. If, by their "deep" expiration (the volume of which they do not state) they have expired for instance 1 litre alveolar air (that is besides the air from the dead space), what they find is then only the difference between $b_1$ and $c_1$, consequently perhaps a difference that is several times lower than the one that matters (that is $a_1 \div d_1$). (In my experiments stated above, I have, as accurately as possible, determined the difference $a_1 \div e_1$, as there is no possibility of calculating $a_1 \div d_1$ by any direct method; so I have come to the results by multiplying $a_1 \div e_1$ by 0·8, on the basis of my previously given calculations.)

3. Even if we assume that **Krogh** and **Lindhard**'s "deep" expiration has really been the deepest possible, the difference found by them will nevertheless only amount to a little more than a half of the one that it is desired to know; thus, when they state a difference of 0·45, as in their most successful determination, the actual difference, even if we grant the most favourable circumstances for **Krogh** and **Lindhard**, will be $0{\cdot}45 \times 2 \times 0{\cdot}8 = 0{\cdot}72$; on subtracting from this 0·25 on account of the exchange during expiration, 0·47 remains as representing the incompleteness of mixing, which, as will easily be seen, for instance in my circulation experiments in Table IV, may involve an error of about 30 %. Even if we ought to subtract considerably more than 0·25 for the exchange, perhaps the double, the error will always be remarkable. As, however, **Krogh** and **Lindhard** have not designated their "deep" expiration as maximum, the possibility remains, as explained above, that the difference found by **Krogh** and **Lindhard** should not only be doubled, but rather tripled or quadrupled, by which the error in the circulation determination in accordance with my experiments, may perhaps amount to about 100 %.

**Krogh** and **Lindhard** advance one more point in support of the efficiency of their method for the circulation rate determination. It is, that **Fridericia** and **Boothby** and **Sandiford**, who have determined the circulation rate by means of the oxygen saturation of the venous and arterial blood, have arrived at identical figures on the same subjects as in **Krogh** and **Lindhard**'s method. They have thus found an oxygen absorption for normal individuals of 50–60 c.c. per litre blood[1]. If, as far as possible, I eliminate the error caused by incomplete mixing in Krogh and Lindhard's equilibrium method, I get however (cf. Table IV) an oxygen absorption of about 100 c.c. But this is not in accordance with the actual facts. The discrepancy may be due to several possible errors, I shall content myself with one, because, in my opinion, it goes far to give a plausible explanation of the fact that the combination of two errors, under certain circumstances, may lead to correct results. For Krogh and Lindhard's method, it is indispensable that the blood during its passage through the capillaries of the lungs becomes perfectly saturated with nitrous oxide to the tension at which the $N_2O$ is found in the alveoli during the pause in respiration. It does not however appear from Krogh and Lindhard's works that they have made sure about this point and it may be doubted whether complete saturation takes place during the short time that is involved.

A. and M. Krogh(3) have found that the oxygen tension of the arterial blood is always lower than that of the alveolar air, usually about 2 % of the atmospheric pressure and in some cases up to 4 %. Whilst conditions for $O_2$ saturation cannot be paralleled with those for $N_2O$ saturation, it will at any rate be obvious that the assumption regarding the $N_2O$ saturation cannot be based upon an analogy with regard to complete saturation in the case of $O_2$ absorption.

If the blood, during its passage through the capillaries of the lungs, does not become completely saturated with nitrous oxide, it will readily be seen, that the error points in the direction of a too high value for the oxygen absorption. If, for instance, we consider the previously mentioned exp. 13, where the $O_2$ absorption by reduction on account of the mixing error has the high value of 93, the blood in this calculation is supposed to be saturated by $\frac{11\cdot73+10\cdot52}{2} = 11\cdot13$ % of the atmospheric pressure, which also represents the average alveolar air tension. If now the saturation of the blood has actually been *e.g.* 4 % less, the $O_2$ absorption should be $93 \cdot \frac{7\cdot13}{11\cdot13} = 63$, that is in fact more in accordance with the actual state of things.

We see then that the efficiency of Krogh and Lindhard's method is not necessarily proved by the fact, that it may, under certain conditions, lead to results that are in accordance with the actual state of things. I maintain that this can only happen because the unquestionable error caused by incomplete mixing occasionally becomes quite eliminated by another error, which may very well be an insufficient $N_2O$ saturation of the blood in the lungs.

---

[1] I have myself determined the circulation rate by means of the carbon dioxide tension of the venous and arterial blood, and found identical relations. *Arch. f. klin. Med.* 127. Nos. 5, 6.

## REFERENCES.

(1) **Krogh and Lindhard.** Journ. of Physiol. 51. p. 59. 1917.

(2) **Sonne.** Pflüger's Arch. 163. p. 75. 1915. Hospitalstidende, No. 43, 1915; No. 9, 1916.

(3) **A. and M. Krogh.** Skand. Arch. f. Physiol. 1910.

# THE EFFECT OF THYRO-PARATHYROIDECTOMY ON THE HEART AND CIRCULATION. BY DAVID BURNS AND ALEXANDER McL. WATSON.

## PART I. ACTION ON NERVOUS CONTROL OF THE HEART.

(*From the Department of Physiology, Glasgow University.*)

ALTHOUGH, practically all who have made observations on dogs after removal of the parathyroid glands, have noted the characteristic tachycardia, no extended research has been carried out on its cause. Falta and Kahn(1) recorded the reaction of the blood-pressure to adrenaline after thyro-parathyroidectomy in one dog. The intravenous injection of 0·75 mg. of adrenaline on the second day after the operation caused a more rapid rise of blood-pressure than in the normal animal. They state that a condition of hyper-excitability of the sympathetic nervous system results from parathyroidectomy. Carlson(2) considers that, in the dog and cat, cardiac activity was not greatly altered by ablation of the parathyroids. He attributes any increase, in rate and strength of the beat during severe tetania parathyreopriva, to the increased muscular activity. Hoskins and Wheelon(3) studied the reaction to adrenaline, nicotine and pituitrine, of dogs, before, and two days after parathyroidectomy. Taking the height of blood-pressure as an index, they found that the action of these drugs was augmented after extirpation of the parathyroids. They conclude that parathyroid destruction, in dogs, results in a marked increase in vasomotor irritability—involving all components of the vasomotor mechanism, sympathetic cells, myoneural junctions and musculature.

It is somewhat dangerous to draw too definite conclusions from experiments in which animals suffering from parathyroid tetany are compared with other apparently normal animals. Caution must also be exercised in deciding how far a change in the working of the cardiac mechanism two days after the operation is primarily dependent upon the removal of the parathyroid glands, and how far it is secondary to the increased muscular activity, alterations in respiration, loss of appetite, etc., which accompany the parathyroid tetany. It appeared to us that the evidence afforded by the work already done in this laboratory(4, 5 and 6) that the symptoms of tetania parathyreopriva are due to the presence of excessive amounts of guanidin and the methyl guanidins in the blood would justify us in investigating whether the action of these substances can in any way explain the tachycardia of tetany.

It may be noted incidentally that(7) McCarrison's theory of the origin of tetany is in no way antagonistic to our results. He maintains that *a* primary cause of tetany is intestinal toxæmia. Thus, one may infer from his work, that the infection of the alimentary canal with certain anaërobic organisms, originating generally from a water supply contaminated with fæcal drainage, results in disease of the thyroparathyroid structure, and the appearance of the symptoms of tetany. In no wise does he controvert our argument that the *cause of the symptoms* following parathyroidectomy, or occurring in idiopathic tetany, is poisoning with guanidin or its methyl derivatives.

Some investigations have been made on the action of guanidin and similar bases on the heart. Putzey and Swaen(8) gave the sulphate of guanidin to frogs, and obtained, first, an increase in rate, followed by a marked slowing of the heart. Jacobj and Hagenberg(9) showed that tetra-methyl ammonium compounds had a muscarin-like action on the frog's heart. Marshall(10) studied the action of quaternary ammonium compounds on the heart of the cat and of the rabbit, both under the influence of anæsthetics, and also on unanæsthetised decerebrate animals. He found, that in the anæsthetised animal tetra-methyl ammonium chloride caused a fall of blood-pressure and a diminution of the frequency of the heart beat. He attributes the fall of pressure to two causes (*a*) vascular dilatation, (*b*) decreased cardiac action. Decerebrated animals differ in reaction from anæsthetised animals in one point only —the fall of blood-pressure is preceded by a rise due, he says, to uncontrolled stimulation of the vasomotor centres. He notes a peripheral muscarin effect and a central stimulation of the vasomotor centre. Fühner(11) pointed out that guanidin and muscarin may be compared in their action to that of the univalent alkaline metals and that the salts of divalent metals like calcium were capable of negativing the action of guanidin and univalent metals alike. He considers that the quaternary ammonium compounds act in the same way as the univalent organic cations. Burn and Dale(12) confirmed Marshall's work. They lay emphasis on the two different types of action shown by methyl-ammonium compounds, *i.e.* a powerful nicotine action sufficient to account for its action on the circulation without having recourse to a hypothetical action on the vasomotor centre etc.—and a muscarin action.

Since the completion of our work, Bovshik(13) has published an abstract of a paper by himself and Sinalnicoff, on the influence of guanidin and its methyl derivatives upon the circulation. They do not state what animals they used, presumably mammals were employed. They obtained with di-methyl guanidin: (*a*) a temporary fall of blood-pressure followed by a long continued rise, accompanied by cardiac retardation; (*b*) on section of the vagi during the period of rising blood-pressure a further increase of the pressure and an acceleration of the heart. This was obtained even after exclusion of all vessels below the diaphragm. They conclude that di-methyl guanidin acts principally on the peripheral nervous and muscular elements of the vessels, and that the existence of vessels of only the upper part of the body is sufficient to increase the pressure. We fail to follow the reasoning by which the latter part of their conclusion is reached. If the lower vessels are excluded by ligature of the abdominal aorta and of the vena cava inf., the administration of the same dose of methyl guanidin per kilo would result in an infinitely greater concentration of the drug in the vessels of the upper part of the body, than would be the case where the whole circulation is open to the drug. They found that mono-methyl guanidin was less active than the di-methyl salt: while guanidin carbonate had no action at all. The concentration of salt used by them was low, viz. 5 mgrms. per kilo. This want of action of guanidin carbonate in small doses is in agreement with the results of Noël Paton and Findlay (*ibid.*) who could detect no alteration in the electrical excitability of rabbits after the intra-muscular administration of this base in amounts under 100 mgrms. per kilo. In order to produce the symptoms of guanidin poisoning these latter workers gave, to cats, from 30 to 120 mgrms. of guanidin hydrochloride per kilo (intra-muscular injections).

The following experiments were planned to determine the action, if any, of the salts of guanidin on the vago-cardiac inhibitory mechanism. The increase of the pulse rate after parathyroidectomy could be explained in one of three ways. Either a block occurred in the path of the inhibitory impulse, or excitation of the accelerator nerves took place, or quickening was due to alterations in vascular tone. Tracings were taken by the method usually employed in this laboratory(14) of the auricular and ventricular beats and of the variations of blood-pressure. All the animals employed were fully anæsthetised except Cat 4 which had been decerebrated by Sherrington's method. In all both carotids were ligatured and a cannula inserted in the left ext. jugular v. for the administration of the drug. The vagi were cut. By means of a Bischhausen (Berne) graduated inductorium and an Edison standard storage battery, the minimal current required to be applied to the R. or L. vagus to produce decided cardiac inhibition was determined before and after the administration of the drug. The salts employed were either the carbonate, hydrochloride or lactate of guanidin and the results were similar in each.

Table I. Minimal effective stimuli in normal animals before and after guanidin and the effect of calcium lactate.

| | | | Before guanidin | | After guanidin | | After Ca lactate | |
|---|---|---|---|---|---|---|---|---|
| Animal | Salt | Dose mgrms. per kilo. | L. | R. | L. | R. | L. | R. |
| Rabbit 1 | Carbonate | 300 | 14 | — | ineffective | — | — | — |
| „ 2 | „ | 300 | 17 | — | „ | — | — | — |
| „ 3 | „ | 300 | 14 | 14 | under 4 | at 14 | 19 | 20 |
| „ 4 | „ | 300 | 14 | 14 | „ 14 | under 14 | — | — |
| Cat 1 | „ | 300 | 14 | 14 | „ 10 | „ 10 | — | — |
| „ 2 | Hydrochloride | 50 | no effect at 0 | 17 | — | „ 16 | — | 16 |
| „ 3 | „ | 100 | „ | 16 | — | „ 16 | — | 16<br>stoppage |
| „ 4 | Carbonate | 300 | 16 | 24 | under 8 | „ 8 | 16 | 24 |
| „ 5 (Decer.) | Lactate | 200 | 18 | 20 | „ 11 | „ 12 | 19 | 20 |
| Dog 1 | „ | 1200 | 20 | — | at 8 | — | 22 | — |
| „ 2 | „ | 1200 | — | — | under 12 | under 12 | 14 | — |

It is obvious that in every case the administration of guanidin salts, in doses of from 50 mgrms. to 1200 mgrms. per kilo, interferes with vagal inhibition of the heart of rabbits, cats and dogs. If the mean of the determinations be taken, we see that the minimal points of stimulation, before the administration of guanidin, were obtained for both vagi with the secondary coil 17 cms. from the primary. After the injection of a salt of guanidin, the coil might be pushed in to 10 cms. without producing a slowing of the heart or a fall of blood-pressure. In six out of the eleven animals, viz., two rabbits, three cats (including the decerebrated cat), and one dog, a short increase of pulse rate resulted from vagal stimulation. Guanidin was given to two parathyroidectomised cats and then the minimal effective stimulus applied to the left vagus. This produced a momentary increase in the amplitude of the ventricle with a slight decrease in the height of the auricular beat. In all cases the administration of a solution of calcium lactate restored the inhibitory mechanism to normal.

To determine whether calcium lactate produces the same effect on parathyroidectomised animals as it does after guanidin observations were made upon parathyroidectomised cats.

TABLE II. Minimal effective stimulus in parathyroidectomised animals and the effect of calcium lactate.

| Animal | Days after p'ectomy | Symptoms | After p'ectomy | | After Ca lactate | |
|---|---|---|---|---|---|---|
| | | | L. | R. | L. | R. |
| Cat 6 | 3 | Depression | 12 | 12 | 14 | 12 |
| „ 7 | 3 | Respir. spasms | 12 | 14 | 20 | — |
| „ 8 | — | Severe depression | 16 | 22 | — | — |
| „ 9 | 5 | Slight tetany | 12 | 14 | 20 | 16 |
| „ 10 | 7 | No symptoms | 20 | 16 | 24 | 24 |
| „ 11 | — | Marked tetany | 14 | 14 | — | — |
| „ 12 | 9 | Slight „ | 12 | 12 | — | — |

Omitting Cat 10 which showed none of the characteristic signs of parathyroid tetany the results may be summarised as follows: after parathyroidectomy the minimal effective stimuli were obtained with the secondary coil at 13 cm. (L. vagus) and 14 cm. (R. vagus). These figures may be compared with the mean (Table I) found for normal cats—viz.—16 and 19 cm. It is worthy of note that the normal animals gave a typical stoppage on vagal stimulation with these strengths, the parathyroidectomised animals merely showed slowing. After the injection of calcium lactate solution, typical inhibition was produced as in normal animals with the secondary coil at 19 and 18 cm. for left and right vagal stimulation respectively. With the exception of Cat 1, section of the vagi caused a slight increase in the pulse rate in parathyroidectomised cats.

Guanidin intoxication and parathyroid tetany produce an interference with vago-cardiac inhibition which is removed by salts of calcium. These two states, guanidin poisoning and parathyroidectomy, differ from one another only in degree.

In order to determine whether guanidin acts on the nerve, on the synapses, on the terminal ganglia or on heart muscle itself, a series of experiments was carried out on twelve frogs. The results are given in Table III.

*A.* After painting the neighbourhood of the sino-auricular junction of the frog's heart with salts of guanidin, stimulation of the medulla with the previous minimal effective stimulus, produced, as in vagal stimulation of the mammal, no inhibition of the heart's action. Stimulation of the crescent with the minimal stimulus effective for the normal heart resulted generally in an increase in the rate of beats. The application of a stronger stimulus produced, first, an increase in rate, then a typical slowing.

TABLE III.

| Frog | Salt and pt of application of guanidin in solutions isotonic with 0·75% NaCl | | Minimal effective stimuli | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Before guanidin | | After guanidin | | | |
| | | | Med. | Cresc. | Med. | | Cresc. | |
| *A.* | | | | | | | | |
| 1 | Cresc. | Carbonate | 8 | 14 | under | 8 | at | 14 |
| 2 | „ | „ | 10 | 18 | „ | 10 | under | 18 |
| 3 | „ | „ | 15 | 17 | „ | 15 | at | 15 |
| 4 | „ | „ | 13 | 17 | „ | 13 | „ | 17 |
| 5 | „ | Lactate | 14 | 14 | „ | 10 | „ | 14 |
| 9 | „ | „ | 12 | 16 | „ | 10 | „ | 14 |
| 10 | „ | „ | 12 | 14 | „ | 10 | „ | 14 |
| *B.* | | | | | | | | |
| 6 | Whole heart | „ | 12 | 16 | at | 12 | Total inhibition. | |
| 8 | „ „ | „ | 13 | 18 | under | 10 | under<br>later at | 10<br>14 |
| I | „ „ | Carbonate | 12 | 12 | „ | 8 | at | 8 |
| II | „ „ | „ | 10 | 12 | „ | 6 | under | 6 |
| III | „ „ | „ | 12 | 12 | „ | 8 | at | 8 |
| 9 (*a*) | „ „ — | Lactate | 12 | 16 | „ | 10 | „ | 12 |

The results are here summarised.

| Before guanidin | | After guanidin (applied about crescent) | | | |
|---|---|---|---|---|---|
| Med. | Cresc. | | Med. | | Cresc. |
| 11 | 15 | under | 10 | at | 14 slight |

Salts of guanidin therefore have a poisoning action on the vago-cardiac synapses, and when painted about the region of the crescent (necessarily in small amount), a slight poisoning effect on the terminal ganglia.

*B.* After painting the whole heart with a guanidin solution, stimulation of the medulla was non-effective whatever the strength of the current, while stimulation of the crescent, in five out of six frogs, produced an acceleration of the heart rate. In two cases the inhibitory path was completely blocked, no stimulus being effective. Three frogs gave a typical cardiac slowing on the application of a stronger stimulus to the crescent.

| Before guanidin | | After guanidin (applied to whole heart) | |
|---|---|---|---|
| Med. | Cresc. | Med. | Cresc. |
| 12 | 14 | under 7 | at 10 |

These results indicate that guanidin has first a nicotine action, poisoning the synapses, and, after more extensive application (larger dose) an atropine action causing paralysis of the terminal ganglia in the heart of the frog.

These results also show that guanidin does not paralyse the sympathetic (accelerator) mechanism in the heart. This is deduced from the quickening of the heart on stimulation of the crescent.

Our best thanks are due to Prof. D. Noël Paton for helpful guidance during the experiments and for kindly criticism both during the experimental period and while the acquired evidence was being weighed.

The cost of these experiments was met by a grant to one of us (D. B.) by the Carnegie Trust, for which we express our thanks.

## REFERENCES.

(1) **Falta and Kahn.** Zeits. f. klin. Med. 74. p. 108. 1912.
(2) **Carlson.** Amer. J. Physiol. 30. p. 309. 1912.
(3) **Hoskins and Wheelon.** Ibid. 34. p. 263. 1914.
(4) **Noël Paton and Findlay.** Quart. J. exp. Physiol. 10. p. 315. 1917.
(5) **Burns and Sharpe.** Ibid. 10. p. 345. 1917.
(6) **Burns.** Ibid. 10. p. 361. 1917.
(7) **McCarrison.** "The Thyroid Gland." London. 1917.
(8) **Putzey and Swaen.** Pflüger's Arch. 12. p. 597. 1876.
(9) **Jacobj and Hagenberg.** Arch. f. exp. Path. u. Pharm. 48. p. 48. 1902.
(10) **Marshall.** (a) Trans. Roy. Soc. Ed. 50. p. 17. 1913.
(b) Ibid. 50. p. 379. 1914.
(c) Ibid. 50. p. 481. 1915.
(11) **Fühner.** Arch. f. exp. Path. u. Pharm. 58. p. 1. 1908.
(12) **Burn and Dale.** J. Pharm. 6. p. 41. 1915.
(13) **Sinalnicoff and Bovshik.** Russki Wrach. No. 23. 1916. (Abstract by Bovshik, Physiol. Abs. 2. p. 358. 1917.)
(14) **Cathcart and Clark.** This Journal, 49. p. 301. 1915.

# THE NUTRITIVE VALUE OF CERTAIN FISH.

BY J. C. DRUMMOND.

*(From the Biochemical Laboratory of the Cancer Hospital Research Institute, London.)*

UNTIL comparatively recently it has been the practice to estimate the nutritive value of foodstuffs merely by a consideration of the relative amounts of the primary food units, protein, fat, carbohydrates, etc., which they contained; this information has been occasionally supplemented by that obtained from digestion and metabolism experiments.

Now, however, that a clearer light has been thrown upon the nature of the requirements for the nutrition of the animal organism, it has become essential that the chief classes of foodstuffs be submitted to a rigorous examination of their true nutritive value as determined by what may be termed the biological method. By this is meant a determination of the influence of the particular foodstuffs upon the processes of growth or maintenance by administering it as a component of the dietary of an animal, the food value of all the other constituents of the ration being already known. By such means a large number of foodstuffs have already been examined, and during the course of these investigations many previously unappreciated deficiencies in food value have been brought to light. McCollum and his co-workers in America have carried out a great deal of pioneer work in this field, particularly in determining the relative nutritive values of the chief foodstuffs of vegetable origin (1). The far reaching importance of the results of such studies cannot be overestimated. An illustration of their application to problems of national food economy may be given in the recent investigation by Halliburton and Drummond (2) of the relative nutritive value of the various types of butter substitutes.

In view of the present position, it is a matter of the greatest importance to ascertain the value of those food-stuffs which will figure most prominently as substitutes for such in the diet of the nation in the near future. Accordingly the question of the true food value of fish becomes one of urgent importance in the national economy.

Unfortunately it has been impossible for many reasons to make the present investigation as comprehensive as was at first desired and the study has therefore been confined to a few types of fish which are consumed in comparatively large quantities in this country.

It is usual to divide the edible fish somewhat empirically into two classes, the so-called "fat" and "lean" fish. The former type are characterised by containing considerable amounts of fat distributed throughout their muscular tissues, whilst the muscle tissue of the representatives of the second class is almost free from fat. Examples of both types are given below:

| *"Fatty" fish* | Approximate % fat | *"Lean" fish* | Approximate % fat |
|---|---|---|---|
| Eel | 14 | Cod | 0·4 |
| Salmon | 13 | Whiting | 0·4 |
| Turbot | 13 | Sole | 0·3 |
| Mackerel | 7 | Haddock | 0·3 |
| Herring | 7 | | |

As considerable quantities of preserved fish are consumed in this country, it was decided to examine one specimen of that type of foodstuff. The fish selected for investigation were:

| | |
|---|---|
| "Fat" fish | Herring (*Clupea harengus*). |
| "Lean" fish | Cod (*Gadus morrhua*). |
| Preserved fish | Salmon (canned) (*Oncorhynchus Chouicha*). |

The following analytical data respecting these fish have been abstracted from the figures given by Atwater(3) and Allen(4).

TABLE I.

| Fish | % Water | % Protein N. × 6·25 | % Protein by difference | % Fat | % Ash | Calories per pound |
|---|---|---|---|---|---|---|
| Cod, steak, edible portion ... ... | 79·7 | 18·7 | 18·6 | 0·5 | 1·2 | 370 |
| Cod, section, edible portion ... | 82·5 | 16·7 | 16·3 | 0·3 | 0·9 | 325 |
| Herring, edible portion ... ... | 72·5 | 19·5 | 18·9 | 7·1 | 1·5 | 660 |
| Salmon, California, anterior section | 63·6 | 17·8 | 17·5 | 17·8 | 1·1 | 1080 |
| Salmon, whole, edible portion ... | 64·6 | 22·0 | 21·2 | 12·8 | 1·4 | 950 |
| Salmon, Californian, canned ... | 63·6 | — | 21·8 | 12·1 | 2·6 | — |

It is mainly the muscular tissue of fish which serves as a foodstuff. The composition of fish muscle has not been much investigated. Osborne and Heyl(5) have published an analysis of the proteins of the muscle of the halibut (*Hippoglossus vulgaris*). Their figures are given in Table II, together with comparative data concerning the composition of ox and chicken muscle published by Osborne and Jones(6).

TABLE II.

| | Ox muscle | Chicken muscle | Halibut muscle |
|---|---|---|---|
| Glycine | 2·1 | 0·7 | 0·0 |
| Alanine | 3·7 | 2·3 | ? |
| Valine | 0·8 | ? | 0·79 |
| Leucine | 11·7 | 11·2 | 10·33 |
| Phenylalanine | 3·2 | 3·6 | 3·04 |
| Tyrosine | 2·2 | 2·2 | 2·39 |
| Proline | 5·8 | 4·8 | 3·17 |
| Aspartic acid | 4·5 | 3·2 | 2·73 |
| Glutamic acid | 15·5 | 16·5 | 10·13 |
| Tryptophane | + | + | + |
| Arginine | 7·5 | 6·5 | 6·34 |
| Lysine | 7·6 | 7·3 | 7·45 |
| Histidine | 1·8 | 2·5 | 2·55 |
| Ammonia | 1·1 | 1·7 | 1·33 |
| | 67·5 | 62·5 | 50·25 |

The distribution of the nitrogen in the proteins of the muscle of the halibut is given in Table III, in which is also given a series of data obtained from the analysis of other types of muscle protein. From these tabulations it will be seen how very similar are the analytical results which indicate the composition of the various muscle tissue proteins.

Preparations of the coagulable proteins of the muscle tissue of herring, cod and salmon were made by the following method. The muscular tissue, separated as completely as possible from other structures, was minced and placed in ten times its weight of distilled water. The whole was heated to boiling point and a few drops of acetic acid were then added to assist the coagulation of the proteins. The latter were filtered off and re-extracted with a further volume of boiling distilled water, again removed by filtration and pressed as free from moisture as possible in a meat-press. The hard cake of almost dry coagulated tissue

TABLE III.

| Protein | % Amide N. | % Humin N. | % Monamino N. | % Diamino N. | Observer |
|---|---|---|---|---|---|
| Chicken muscle | 7·49 | 2·74 | 60·00 | 30·00 | Osborne and Heyl (5) |
| Ox muscle | 5·51 | 2·66 | 64·40 | 27·30 | Osborne and Jones (6) |
| Halibut muscle | 6·70 | 2·37 | 60·80 | 30·20 | Osborne and Heyl (5) |
| Human muscle | 6·30 | 2·30 | 62·25 | 29·90 | Drummond (7) |
| Chicken muscle | 6·63 | 1·76 | 61·55 | 30·27 | " |
| Cod muscle | 5·82 | 2·30 | 61·20 | 30·00 | Drummond (this study) |
| Herring muscle | 6·05 | 2·64 | 61·40 | 29·70 | " " " |
| Salmon (canned) muscle | 5·94 | 2·67 | 60·70 | 29·70 | " " " |

was broken up and dried by two extractions with hot alcohol. Finally the residue was powdered, passed through a fine mesh sieve, and subjected to a prolonged ether extraction. The preparations obtained by this process were pale sandy-coloured powders. Samples were submitted to a determination of the distribution of nitrogen in four groups by a modified Hausmann method. The results are given in Table III. The aqueous extracts from the coagulated tissue contained a considerable amount of a substance resembling gelatin.

The tissues of fish are comparatively much richer in this type of substance than the ordinary meat foods. The amount of the so-called gelatinoids present in the flesh of a number of fish has been estimated by Atwater(3), and certain results obtained by him are given in Table IV.

TABLE IV. Composition of water-free substance of fish. Atwater (3).

| Fish | % Non-coagulated matter in cold water extract | % Coagulated protein in cold water extract | %-Gelatin substances in hot water extract | % Insoluble protein | % Ash | % Fat |
|---|---|---|---|---|---|---|
| Herring | 4·51§ | 5·23 | 9·46§ | — | 4·83 | 35·55 |
| Canned salmon | 14·21† | — | 5·27† | 42·44† | 5·24 | 32·40 |
| Haddock* | 6·18† | 7·89 | 16·36† | 65·06 | 8·72 | 0·78 |

* No analysis of cod is recorded by Atwater. Haddock however bears a close resemblance, being of the same type.

§ Calculated upon ash and fat-free basis.

† Calculated upon ash-free basis.

Upon concentration of the neutralised aqueous extracts of the coagulated fish tissues at 40° under reduced pressure a residue was obtained which on cooling set to a firm jelly. This was re-dissolved in a small quantity of distilled water and the solution poured into ten volumes of alcohol. A flocculent white precipitate was obtained, which on being allowed to settle overnight, formed a somewhat sticky mass. This was removed and re-dissolved in a small quantity of cold distilled water, the solution filtered and again poured into ten times its volume of alcohol. This time the precipitate was less sticky and settled more readily. It was removed by filtration and again reprecipitated from aqueous solution by means of alcohol. The resulting white precipitate was now in the form of a powder and was readily filtered off on a Buchner funnel, well washed with alcohol and finally with ether. The dry product was a light white powder, slowly soluble in cold water and contained a considerable amount of inorganic impurity. It gave reactions similar to those described for gelatin. It yielded no positive reactions for tryptophane and only a very faint coloration was obtained with Millon's reagent. The substance probably arises from the connective tissue, which is also a probable source of the so-called "reducing substances" which have been described as being present in fish (Williams(8)).

### *The nutritive value of the coagulable proteins of fish.*

The most reliable method now available for determining the value of a protein as a foodstuff is by studying the effect of administering it at various levels of intake as a component of an otherwise adequate diet. By such experimental means it has been found possible to assign to many pure proteins their comparative biological value for growth or maintenance (Osborne and Mendel(9)).

Somewhat similar lines of investigations have been followed during the present research. The preparations of fish tissue proteins were administered at various levels as the sole source of protein nitrogen in the diet of young rats, and the resulting effect upon the nutrition of the young animal was compared with that produced by a similar ration containing an equivalent proportion of a protein whose nutritive value was known. At the same time, for comparative purposes, the nutritive value of the coagulated proteins of ox muscle—prepared exactly as has been described for the coagulated fish proteins—was studied. The rations were prepared as follows:

| | | | | |
|---|---|---|---|---|
| **Protein** ... ... ... ... ... | **6** | **10** | **15** | **18** |
| **Starch** ... ... ... ... ... ... | **64** | **60** | **55** | **52** |
| **Salt mixture** ... ... ... ... ... | **5** | **5** | **5** | **5** |
| **Fat with fat-soluble accessory substance** ... | **20** | **20** | **20** | **20** |
| **Yeast extract** ... ... ... ... ... | **5** | **5** | **5** | **5** |

The composition of the salt mixture was given in a previous communication (2).

Owing to the present scarcity of butter it was necessary to employ another fat for inclusion in the rations. The choice of an edible fat for this purpose was of course limited to those which are known to contain an adequate amount of the fat-soluble accessory substance. During the earlier part of the work no difficulty was encountered as beef fat was available, but when the supply of this product also ran short, it was necessary to fall back on cod-liver oil, which was, however, from some points of view, found to be somewhat less satisfactory than either of the other fats.

The proteins investigated were caseinogen, ox muscle protein, cod muscle protein, herring muscle protein and the protein of the muscle of the canned salmon. In all cases the diets were consumed satisfactorily by the experimental animals.

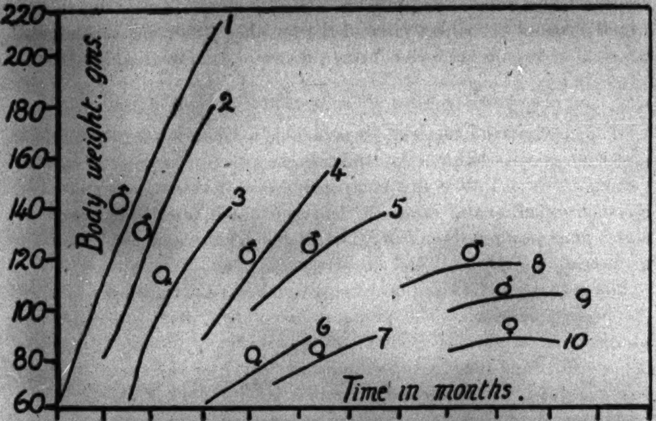


Fig. 1. Curves 1–3, growth upon ration containing 18 p.c. caseinogen. Curves 4–7, growth upon ration containing 10 p.c. caseinogen. Curves 8–10, growth upon ration containing 6 p.c. caseinogen.

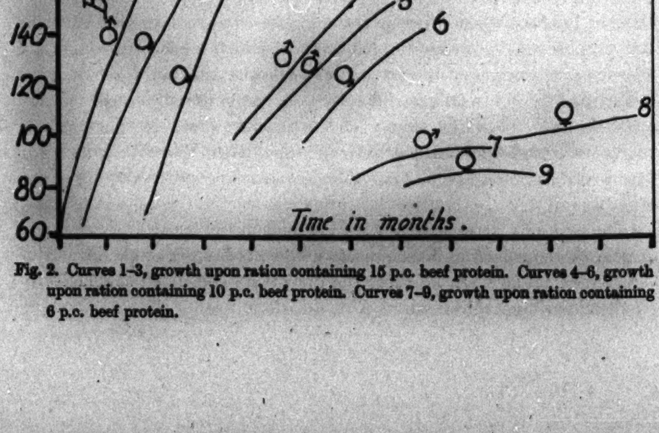


Fig. 2. Curves 1–3, growth upon ration containing 15 p.c. beef protein. Curves 4–6, growth upon ration containing 10 p.c. beef protein. Curves 7–9, growth upon ration containing 6 p.c. beef protein.

Fig. 1 shows the growth curves of a number of rats fed upon the caseinogen rations. Curves 1–3 illustrate a normal rate of growth upon the diet containing 10 p.c. of caseinogen. Curves 4–7 demonstrate that the lower protein intake upon the 10 p.c. caseinogen ration brings about a retardation of the rate of growth, whilst little growth at all was accomplished upon the still lower intake of the 6 p.c. ration (Curves 8–10). Caseinogen has been shown to possess a relatively high value as a source of protein to the growing animal, but its low cystine content may operate at low levels of intake as an inhibitory factor for growth. Lactalbumin, however, possesses an unusually high value (Osborne and Mendel(9)).

Fig. 2 shows the results obtained by feeding young rats upon rations containing the coagulated proteins of ox muscle as a sole source of protein nitrogen. Normal growth was obtained upon a ration containing 15 p.c. of this protein (Curves 1–3), whilst with 10 p.c. (Curves 4–6) and 6 p.c. (Curves 7–9) the results were very similar to those obtained with the corresponding caseinogen ration.

Osborne and Mendel have recently(10) shown that the proteins of beef and pig's liver are efficient sources of nitrogen for the nutrition of young animals during growth. The results obtained upon feeding the young rats upon the various fish protein dietaries are given graphically in Figs. 3 and 4. It will be seen from a glance at these figures that the coagulable proteins of the fishes examined possess a nutritive value quite as high as those derived from beef. In considering these results in the light of their application to problems of practical nutrition it must be remembered, however, that fish muscle is relatively poorer in protein than beef and the majority of other mammalian muscles, whilst the proportion of substances of the gelatin type which possess a low nutritive value, is higher in the case of fish. The nutritive value of the total protein present in equal weights of fish and beef is therefore probably somewhat higher in the case of the latter food. It is also important that due consideration be given to the relative digestibility of the various types of flesh foods. The present investigation has only attempted an examination of the value of the proteins of the tissues for nutrition, when they were administered in a condition which must be regarded as artificial. That is, they had been freed from fat and extractive substances, dried and reduced to fine powders. The nutritive value of a foodstuff is necessarily governed to a very large extent by its digestibility, and certain of the chief fish foodstuffs are considered to be digested with a certain amount of difficulty, some such as salmon owing to the presence

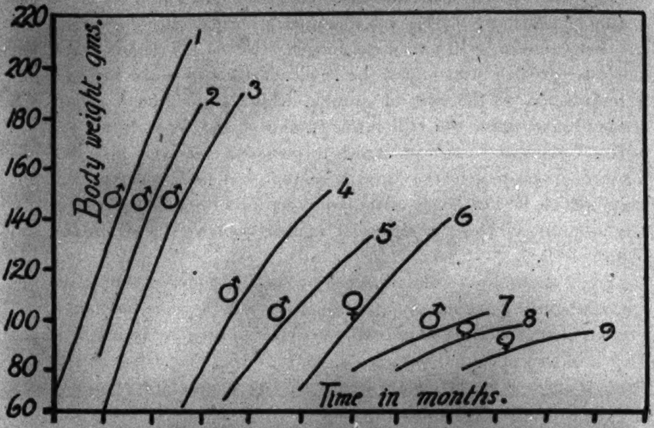


Fig. 3. Curves 1–3, growth upon ration containing 15 p.c. herring protein. Curves 4–6, growth upon ration containing 10 p.c. herring protein. Curves 7–9, growth upon ration containing 6 p.c. herring protein.

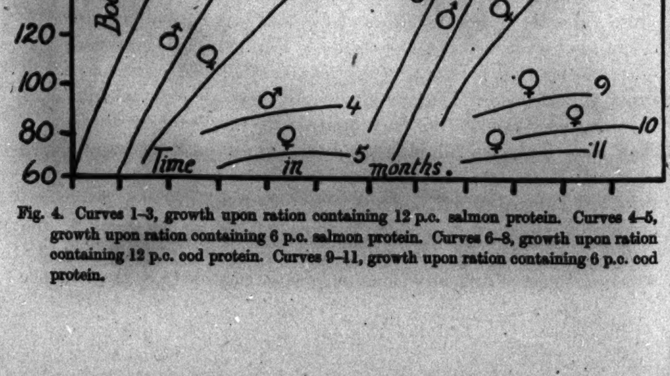


Fig. 4. Curves 1–3, growth upon ration containing 12 p.c. salmon protein. Curves 4–5, growth upon ration containing 6 p.c. salmon protein. Curves 6–8, growth upon ration containing 12 p.c. cod protein. Curves 9–11, growth upon ration containing 6 p.c. cod protein.

of considerable amounts of fat, others, such as cod, owing to the coarseness of the muscle fibres. It is therefore necessary to give all such facts as these due consideration before finally adjudging the value of any particular fish as a source of protein for the animal organism.

### *Concerning the occurrence of the fat-soluble accessory substance in the fats of fish.*

A very great interest centres at the present time round the substance, or substances, present in certain fats and oils which exerts a beneficial influence upon the growth and health of the animal organism. This factor has been termed by McCollum "the fat-soluble *A*." Cod-liver oil has been shown to contain this substance(11), and it is probable that the valuable nutritive powers of this oil are to a large extent due to its content of the fat-soluble accessory factor. It is unfortunate that the majority of fish oils—which may be broadly classified into body oils and liver oils—are very unpalatable, by reason of their unpleasant taste and odour, for it appears to be probable from this study that the majority of them contain the fat-soluble accessory factor. This is particularly so in the case of the liver oils which appear to be very rich in this unidentified substance. It is of interest to note that Bloch(12) and Monrad (13) have observed a condition in young children which is apparently due to their having been reared upon dietaries deficient in the fat-soluble factor. The symptoms described by them are closely similar to those which may be observed in young rats fed upon diets showing that deficiency. Particularly characteristic is the condition of xerophthalmia, which occurs with great frequency in these cases.

Mori(14) had previously observed a large number of cases in Japan, during times of famine, showing eye troubles similar to those which are observed in young rats fed upon rations deficient in the fat-soluble accessory. He attributed the condition to fat starvation, as the existence of the accessory substances was then unknown, but he records the interesting fact that chicken liver and eel fat have for years been used as domestic measures against the complaint. Many fish oils and fish-liver oils were found to be curative, and this is held to account for the relative scarcity of the disease amongst the fisher families. The following fish oils and fish-liver oils were tested for the presence of the fat-soluble factor, and I must here express my great indebtedness to Mr F. Weis, for his kindness in placing many of the samples at my disposal.

TABLE V.

| Oil | Remarks |
|---|---|
| Salmon oil. | Ether extract of dried canned salmon, prepared during this investigation. Deep red oil, closely similar in properties to the salmon oil of commerce. |
| Herring oil. I. | Ether extract of dried herring tissue, prepared during the course of this investigation. |
| Japan herring oil. | An oil prepared by digesting the flesh of herrings with steam and skimming off the oil. |
| Menhaden oil. | Commercial oil prepared by steam digestion of the flesh of an American fish resembling the herring (*Alosa Menhaden*). |
| Cochin fish oil. | Similar to Japan herring oil. |
| Cod oil. | A Norwegian oil prepared from the bodies of the cod after removal of the livers. |
| Crude cod-liver oil. | A "black" Norwegian oil, representing the crude oil obtained by digestion of the partially decomposed livers after extraction of the finer qualities of oil. |
| Raw medicinal cod-liver oil. | The finest liver oil prepared by allowing the oil to exude from the fresh livers. |
| Shark-liver oil. | A preparation of the moderately refined oil. Prepared by steaming the fresh shark livers. |
| Whale oil. | An oil known technically as of No. 3 quality. Such an oil represents the very crude product obtained by digestion of the carcase after all the blubber has been removed. |

Twenty parts of the various oils were added to a basal diet composed is given below:

| | | | |
|---|---|---|---|
| Caseinogen | 18 parts | Salt mixture | 5 parts |
| Starch | 52 „ | Yeast extract | 5 „ |

The resulting dietaries were administered to batches of young rats.

When the ration contains a fat (such as lard or many of the vegetable oils), which is deficient in the fat-soluble accessory substance, the young rats are able to grow for a short period, but the rate of growth soon shows a falling off and the health of the animal declines. Examples of this may be seen from Curves 1–4 and 10 in Fig. 5, which show the response of young rats fed upon a diet containing coconut oil, a fat deficient in the accessory factor. Three of these five animals developed a sore condition of the eyes towards the end of the trial. Curves 5–9 of the same figure show the effect when Menhaden oil replaced the coconut oil in the ration. These five rats showed very little growth, but they did not at any time show signs of the xerophthalmia, which may be considered to be a characteristic symptom following an entire lack of the accessory substance. These animals were also enabled to maintain a very fair standard of health throughout the experiment, an observation

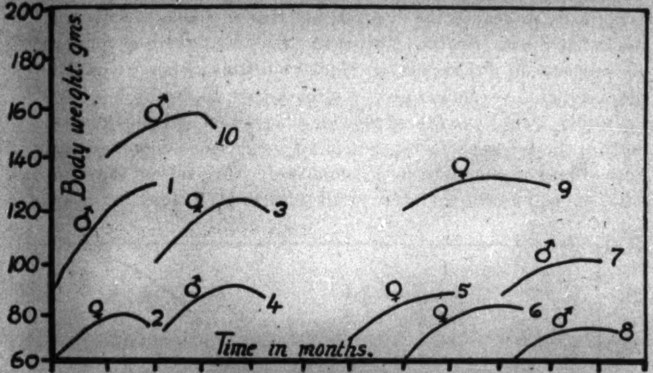


Fig. 5. Curves 1–4 and 10, growth upon diet deficient in "fat-soluable A" (coconut oil diet). Curves 5–9, growth upon 20 p.c. Menhaden oil diet.

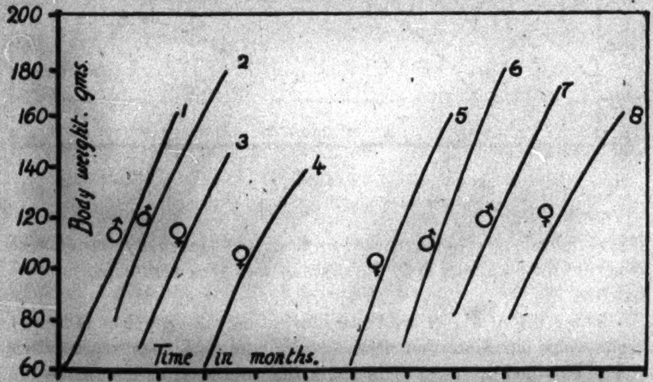


Fig. 6. Curves 1–4, growth upon adequate diet containing 20 p.c. of crude "black" cod-liver oil. Curves 5–8, growth upon dietary containing 20 p.c. of refined "white" cod-liver oil.

which indicates that the particular oil contained the factor, but in too small an amount to permit appreciable growth.

From Fig. 6 it may be seen that both the refined and the crude cod-liver oils are adequate sources of the unidentified factor. This indicates not only that the substance responsible for the growth stimulating action is normally present in the liver fat, but that it is able to withstand the somewhat drastic treatment by steam digestion, which is employed in the preparation of the crude oil. Similar but less satisfactory results were obtained on the examination of the shark-liver oil.

In Fig. 7 are given curves showing that cod oil and Japan herring oil contain the accessory factor, but in an insufficient amount to enable a normal rate of growth to be accomplished. Nevertheless these animals enjoyed good health, and were at all times in good condition in spite of being undersized. Similar curves to these were given by the animals receiving the cochin oil diet. No cases of sore eyes were noted in these batches.

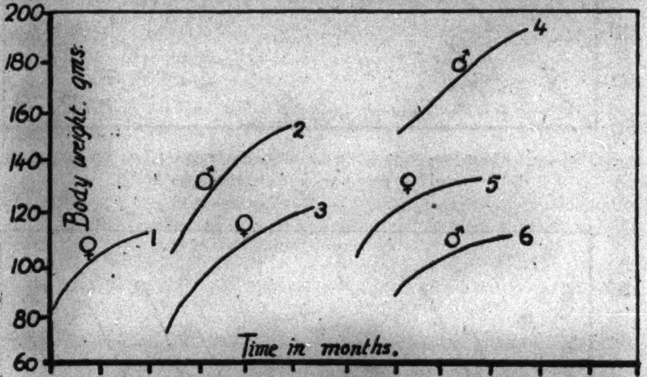


Fig. 7. Curves 1–3, growth upon diet containing 20 p.c. cod oil. Curves 4–6, growth upon diet containing 20 p.c. Japan herring oil.

Curves 1–4 in Fig. 8 are those illustrating the growth influence of crude whale oil. Since this oil was a very crude specimen, representing the oil obtained by steam digestion of the carcase, after removal of the blubber, it is probable that it contained substances derived from the liver. Whether this is so or not, it is at any rate apparent that the crude whale oil possesses a very satisfactory growth promoting action. In Fig. 8 are given Curves 5–7 and 8–10, which illustrate respectively the growth promoting actions possessed by the ether extract of dried salmon and herring muscle tissue. Although the rate of growth of the animals fed upon these rations is subnormal, there is unmistakeable evidence of the presence of the fat-soluble accessory substance.

### *Experiments to ascertain whether fish may serve as a source of the water-soluble factor in the diet.*

With one or two exceptions animal tissues appear to be deficient in the water-soluble antineuritic factor. The chief exceptions are the pancreas and the liver, both of which have been reported to contain this substance (15, 10). Concentrated, protein-free, aqueous extracts of the muscle tissue of the three types of fish investigated in this study were found to possess no power to serve as sources of the water-soluble factor (Table VI). Feeding with the fresh muscle tissue gave the same results.

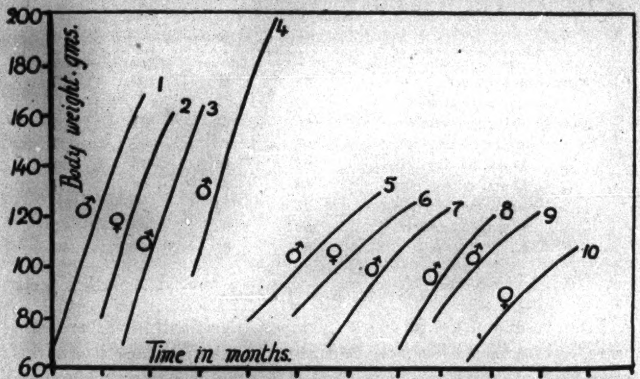


Fig. 8. Curves 1–4, growth upon diet containing 20 p.c. crude whale oil. Curves 5–7, growth upon diet containing 12 p.c. ether extract salmon. Curves 8–10, growth upon diet containing 12 p.c. ether extract herring.

A concentrated, protein-free, aqueous extract of the whole herring apparently contained a small amount of the unidentified substance, as young rats were enabled to show slight growth when fed upon a ration, adequate except for a deficiency of the water-soluble factor, to which this extract was added. This result may be due to the extract containing substances derived from tissues such as the reproductive organs, for Chick and Hume (16) have recorded that the ova of fish (turbot) contain the antineuritic "vitamine," and it is highly probable that that substance is identical with the so-called water-soluble *B*(17). The above results were obtained by adding the various extracts and tissues to an artificial dietary, deficient in the water-soluble factor, but adequate in other respects and composed as follows:

| | |
|---|---|
| Caseinogen ... ... ... ... ... ... | 18 parts |
| Starch ... ... ... ... ... ... ... | 57 „ |
| Fat (containing fat-soluble accessory substance) ... | 20 „ |
| Salt mixture ... ... ... ... ... ... | 5 „ |

The results themselves are given in Table VI.

TABLE VI.

| Substance added to 100 g. of inadequate ration | Average weight of 6 rats | | | |
|---|---|---|---|---|
| | 1st day | 11th day | 21st day | 31st day |
| 5 gms. yeast extract | 62 | 86 | 100 | 127 |
| Concentrated aqueous | | | | |
| Extract 70 gm. cod muscle | 66 | 68 | 61 | 57 |
| „ 62 gm. herring muscle | 56 | 64 | 60 | 57 |
| „ 54 gm. herring, whole | 63 | 69 | 76 | 82 |
| „ 61 gm. salmon muscle | 58 | 58 | 56 | 51 |
| „ 10 gm. herring muscle | 76 | 74 | 70 | 66 |
| „ 20 gm. cod muscle | 68 | 62 | 60 | 53 |
| nil | 60 | 61 | 58 | 54 |

## *Consideration of Results.*

From results of the feeding experiments described in the preceding pages, certain conclusions may be drawn. In the first place, it is at once apparent that the coagulable proteins of fish muscle possess a high value as a source of protein to supply the requirements of the organism for growth or maintenance, in fact it appears probable that they are as satisfactory for this purpose as are the proteins of beef. In view of the present shortage of butcher's meat this is an important fact which may, if taken advantage of, be the means of effecting considerable economies in the national requirements for protein.

When we consider the nutritive value of the so-called "fatty" fishes, it must be remembered that not only do they serve as valuable sources of protein and of fat, but that they are also sources of the very important accessory factor, the fat-soluble "*A*."

Halliburton and Drummond(2) recently pointed out that there was a danger of the dietaries of the poorer classes being deficient in this particular factor, owing to the shortage and high prices of the dairy products—the chief sources of the fat-soluble "*A*." In the light of the present experiments it would appear that this danger is unlikely to arise whilst there is a cheap and plentiful supply of the "fatty" fishes, such as the herring. The very high nutritive value of this particular fish cannot be over emphasised.

## SUMMARY.

1. The coagulable proteins of the muscle tissue of cod, herring and canned salmon possess a nutritive value as high as those derived from beef.

2. The so-called "fatty" fish, which contain considerable quantities of fat distributed throughout their muscle tissue, may serve as valuable sources of the important dietary essential, the fat-soluble "*A*."

3. Certain fish-liver oils are particularly rich in the fat-soluble accessory.

4. No appreciable amounts of the water-soluble or antineuritic factor were detected in the muscle tissues of the fishes examined. Small amounts were, however, present in extracts prepared from the whole herring, having originated in all probability from the reproductive organs, or other glandular organs.

A considerable portion of the expenses of this research were defrayed from a grant made to Professor Halliburton by the Government Grant Committee of the Royal Society.

## REFERENCES.

(1) **McCollum and Simmonds.** Journ. Biol. Chem. **32**. p. 29. 1917.
(2) **Halliburton and Drummond.** Journ. Physiol. **51**. p. 235. 1917.
(3) **Atwater.** The chemical composition and nutritive values of Food Fishes, Washington. 1891.
(4) **Allen.** Commercial Organic Analysis, **8**. London. 1914.
(5) **Osborne and Heyl.** Amer. Journ. Physiol. **23**. p. 81. 1908.
(6) **Osborne and Jones.** Ibid. **24**. p. 437. 1909.
(7) **Drummond.** Biochem. J. **10**. p. 473. 1916.
(8) **Williams.** J. Chem. Soc. **71**. p. 649. 1897.
(9) **Osborne and Mendel.** Jour. Biol. Chem. **26**. p. 1. 1916.
(10) **Osborne and Mendel.** Ibid. **32**. p. 309. 1917.
(11) **Osborne and Mendel.** Ibid. **17**. p. 401. 1914.
(12) **Bloch.** Ugesk f. Laegar. **79**. p. 349. 1917.
(13) **Monrad.** Ibid. **79**. p. 1177. 1917.
(14) **Mori.** Jahrbuch f. Kinderheilkunde, **59**. p. 175. 1904.
(15) **Eddy.** Journ. Biol. Chem. **27**. p. 113. 1916.
(16) **Chick and Hume.** Journ. R.A.M.C. **29**. p. 121. 1917.
(17) **Drummond.** Biochem. Journ. **11**. p. 255. 1917.

# THE VASODILATOR ACTION OF HISTAMINE AND OF SOME OTHER SUBSTANCES. BY H. H. DALE AND A. N. RICHARDS.

*(From the Department of Biochemistry and Pharmacology of the Medical Research Committee, at the Lister Institute, London, S.W.)*

CONTENTS.

PAGE
I. Methods . . . . . . . . . . . . 112
II. Mautner and Pick's theory . . . . . . . 115
III. Plethysmographic records with normal circulation . . . 118
  (i) Direct nature of the vasodilator effects . . . 118
  (ii) Volume-changes in the normally innervated leg . 121
  (iii) Immediate effects of nerve section . . . 125
  (iv) Effect of complete denervation . . . . 130
  (v) Effects of prolonged anæmia and cooling . . 136
IV. Distribution of the vasodilator action . . . . 139
V. Effects of local application of histamine . . . . 143
VI. Perfusion of surviving organs . . . . . . 144
VII. Remarks . . . . . . . . . . . . 159

THE immediate object of the experiments with which this paper deals was a more complete analysis of the vasodilator effect produced, in some species of animals, by small intravenous doses of the substance which we have here called histamine. In earlier papers P. P. Laidlaw and one of us(1) referred to it by its chemical name, β-Iminazolylethylamine (abbreviated for convenience to β-I.), being unwilling to coin a new name for a substance long known, though its great physiological activity and occurrence in nature were at that time newly discovered. Several later investigators of its action, however, have used for it the name "histamine," which is so obviously appropriate for this amine derived from histidine, that we have adopted it here.

The most puzzling feature of the action of histamine, as described by Dale and Laidlaw, was the fact that this intense stimulant of plain muscle caused in the carnivora, and also in the monkey and the fowl, a profound fall of the arterial blood-pressure in the major circulation, due apparently to pure vasodilatation. Plain muscle from any organ of the body of various species was found to be stimulated to intense tonus. A solitary and curious exception has since been found by Guggenheim(2) in the plain muscle of the rat's uterus. In some species, such as the rabbit, histamine caused a vasoconstrictor rise of arterial pressure, such as its property of stimulating plain muscle would lead one to expect. Even in the cat a vasoconstrictor action could be detected in the pulmonary circulation, and in the kidney; but the general effect in this species was vasodilatation, so long as the injection was made into the blood stream of the living, anæsthetised animal. In surviving organs of the cat, on the other hand, perfused with defibrinated blood, histamine was found always to cause simple vasoconstriction, as demonstrated by the record of venous outflow and by the plethysmograph. Later observers have shown that isolated strips of artery, from various organs, are always stimulated to increased tonus by application of histamine.

Dale and Laidlaw considered and tested the more obvious possibilities of explaining this vasodilator action, paradoxically produced by a general stimulant of plain muscle, but left the problem unsolved. The numerous papers dealing with the action of histamine which have since appeared have not materially affected this position, being concerned, for the most part, with detailed investigation on particular organs of an action in conformity with the general type indicated. An attempt has been made, however, by Mautner and Pick(3) to account for the discrepancy in action with which we are concerned, and we shall deal with this later.

The immediate impulse to a renewed investigation of this anomalous vasodilator action of histamine was furnished by the observation that this substance in larger doses produces, in the cat or dog, a condition of profound shock, having many of the features which recent work has associated with shock of traumatic origin. This shock-like effect of large doses of histamine has been described by E. Mellanby(4), who investigated especially the conditions determining its production by absorption of histamine from the bowel. A preliminary account of a further analysis of the condition by Dale and Laidlaw(5) has already been published, and fuller details will be given in a forthcoming paper. The shock-like condition caused by large doses seemed to be definitely associated with the vasodilatation which small doses produce in an evanescent form; the one seemed to result from a prolongation and intensification of the other, and both types of action, if indeed they were separate, were observed only in certain and the same species. It seemed to us, therefore, that a further attempt to analyse and explain the vasodilator action of small doses was a necessary part of the investigation of the shock-like conditions produced by larger doses, which in turn might be expected to throw light, if only by inference, on the nature of the circulatory defect characteristic of traumatic shock and allied conditions, at present of such pressing interest and importance.

Incidentally the opportunity has occurred of examining, in comparison with that of histamine, the vasodilator effects of certain other substances, in particular those of minute doses of adrenaline and of acetyl-choline. Our experiments lead us to reject the conception recently put forward by **Hartmann** and **Fraser** (6), according to which the vasodilator effect produced by very small doses of adrenaline in the cat and dog is an indirect effect, due to action on the nervous centres. In the case of acetyl-choline we give additional evidence concerning the vasodilator action described by one of us (7) (H. H. D.), and recently confirmed by **Reid Hunt** (8), who first described the intense depressor action of this substance. We find that this action can be demonstrated in conditions under which the vasodilator effect of histamine no longer appears, and the difference furnishes an important link in our evidence concerning the mode of action of the latter substance.

## I. METHODS.

All our experiments have been made upon cats, except where otherwise stated. Ether has been the anæsthetic regularly used, chloroform being sometimes used for the preliminary induction. In the experiments in which observations have been made on denervated organs the nerve sections have been carried out under ether, with full precautions against sepsis. After the requisite period for degeneration the main observation has been made under ether in the usual manner. Occasionally the progress of sensitiveness to histamine has been tested, during the degeneration of nerves to a limb, by injecting a small dose of histamine into the ear-vein and directly observing the vasomotor changes in the unpigmented pads of the feet; or, again, such an interim test has been made with the aid of plethysmographs on the legs, the animal being then immobilised by full ether anæsthesia and injections made into the ear-vein or into the jugular vein exposed aseptically by a very small skin-incision. After such an interim experiment, the animal has been allowed to recover from the anæsthetic, but after the main experiment, involving dissection for the record of blood-pressure, etc., the animal was always killed while still anæsthetised.

Our experiments fall into two groups: those made on the animal with natural circulation, and those made on surviving organs perfused artificially. In experiments of both kinds the plethysmographic method was frequently employed. For the viscera we used air-oncometers of the usual type, shaped out of gutta-percha, and closed by glass lids made tight with vaseline. For the limbs we used cylindrical glass plethysmographs filled with warm water (or with saline in experiments on exposed muscle). The method of making a water-tight junction between the skin of the limb and the end of the plethysmograph deserves mention, as we have found it unusually convenient and trustworthy.

The glass cylinder is made considerably wider than the thickest part of the limb to be enclosed, and the water-tight junction is, as usual, effected by use of a suitable cuff of thin rubber. A section of inner tube from a bicycle tyre serves well for the legs of most cats. The only point of novelty in our use of the cuff is that we invaginate its free portion into the open end of the glass cylinder, within which it forms a secondary, short elastic tube, separated from the glass by an annular space, a centimetre or so in breadth (Fig. 1). The rubber is well softened with vaseline, and the limb, at the level at which the connexion is to be made, is well anointed with the same substance, which is thoroughly incorporated with the fur. The plethysmograph, with its invaginated cuff, is simply pushed onto the limb, and an air-tight fit is usually secured immediately. If the limb is very thin it can be built up to the requisite thickness with vaseline-soaked cotton-wool, but this is seldom needed. When the junction has been tested the cylinder is filled, except for a small air-space at its distal end, with warm water. The water fills the annular space between glass cylinder and rubber cuff, the warm water softens the vaseline, and the small hydrostatic pressure, produced by giving the

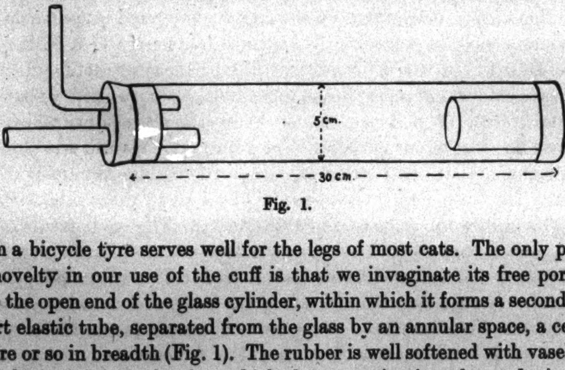


Fig. 1.

apparatus a slight upward inclination, keeps the rubber gently pressed against the vaselined fur. The plethysmograph is then fixed in position by a condenser-clamp and the temperature of the water in it kept constant by means of a carbon filament glow-lamp placed near to it. When once this form of plethysmograph has been satisfactorily applied,—a procedure which seldom occupies more than a few minutes—it gives a very satisfactory safety from leakage without any undue pressure on the limb by the cuff. The leg can be raised with the plethysmograph to any required angle, even to the vertical, and the cylinder of the plethysmograph can be displaced along the length of the leg through several centimetres, without a leak occurring.

As volume-recorders we have throughout used small Brodie's bellows, covered with the prepared peritoneal membrane sold for surgical use as "Cargile membrane." Recorders made with this membrane, when kept supple by frequent application of dilute glycerine, have remained in almost daily use for months without leaking or giving trouble. For the perfusion of surviving organs we should have preferred to use an adjustable pump, such as that recently described by one of us (A. N. R.) with C. K. Drinker(9). Recent conditions making the construction of this or any suitable form of pump for us impossible, we have used a relatively simple system of perfusion under hydrostatic pressure, which has sufficed for most of our purposes. The adjustable pressure was obtained by slinging a Marriotte-bottle from the ceiling by a cord passing over a pulley. The outflow tube was connected by a long rubber tube with one end of a glass worm immersed in a water-bath. The temperature of the water was kept approximately constant by a long cylindrical glow-lamp with carbon filament, which could be inserted to any required depth into a metal tube, soldered into the side of the bath, and closed at its inner end. From the other end of the worm connection was made by rubber tubing to the arterial inflow-cannula, a glass T-piece being interposed for connection with a mercury manometer recording the perfusion pressure. The pressure was given a pulsatile character by the rhythmical compression of the rubber tube between the worm and the cannula by a small hammer driven by an electro-magnet. The electro-magnet was actuated by the house-current with a lamp resistance, the circuit being momentarily made with the desired rhythm by closure of a brass contact key by a rotating vulcanite cam.

As perfusion fluids we used chiefly hirudinised cats' blood, and oxygenated Locke-Ringer's fluid to which the requisite viscosity was imparted by adding 6 p.c. of gum arabic freed from calcium, according to the recent indications of Bayliss(10), who showed that such an addition also supplied the required amount of osmotically active colloid. In two experiments we used a lower proportion of gum in the Ringer solution and suspended washed corpuscles from cats' blood in the mixture. In one case we obtained a result before agglutination of the corpuscles interfered with the perfusion; in the other case the presence of the gum caused such rapid agglutination of the corpuscles that the experiment had to be abandoned. Bayliss has observed that the red corpuscles of the cat, unlike those of man, are agglutinated by gum-arabic.

The rate of outflow from the cannula in the vein leading from the perfused organ was measured in all experiments. In earlier experiments we used an electrically recording tilter for automatic registration of the rate, but more recently we allowed the outflowing blood or fluid to run into a measuring burette and recorded the completion of each c.c. by an electric signal. From time to time the fluid collected from the vein was returned to the Marriotte-bottle. The lack of apparatus to effect this return automatically necessitated the use of rather large volumes of fluid, to prevent too frequent interruptions of the record; so that when blood was used we generally employed the whole blood of two cats for an experiment.

## II. MAUTNER AND PICK'S THEORY.

The theory of Mautner and Pick, already mentioned above, is stated in what appears to be a preliminary communication, since no quantitative details are given. Their experiments were carried out on artificially perfused livers and lungs of various species. They found that histamine and other substances belonging to the group which they denominate "shock-poisons" produced obstruction to the perfusion through the portal circulation of the cat and dog. In these species, therefore, they regard the fall of arterial pressure as due to the obstruction of blood-flow through the liver. Since the liver expands as the outflow through the hepatic veins diminishes, they locate the obstruction in the capillaries opening into the intralobular veins. Such part of the depressor action as is not explained by this obstruction in the liver—presumably the whole of the depressor effect in the monkey, in which they detected no impediment to the liver-circulation—they attribute to constriction of the pulmonary vessels. The possibility of the fall of systemic arterial pressure in the cat being a result of vasoconstrictor action on the pulmonary vessels was considered by Dale and Laidlaw, who showed that such an explanation was inconsistent with the facts. Among other points of evidence against it they mentioned the fact that the fall of arterial pressure has a shorter latency when histamine is injected into the arterial blood-stream than when it is injected into a vein.

In attributing such general significance to the obstruction in the liver of the cat and dog, as an explanation of the depressor action in these species, it seems to us that Mautner and Pick have too readily assumed that all the effects of all doses of the "shock-poisons" can be explained by one feature of their action in massive doses. Nobody who has watched the exposed abdominal viscera of a dog after a sufficient dose of peptone has been injected to produce "peptone-shock," or during the closely similar "anaphylactic-shock," can be in doubt as to the large part played in the effect by obstruction to the circulation through the liver.

Fig. 2. Effects of intra-aortic, intracaval and intraportal injections of 0·01 mgm. histamine.

The appearance of the enormously swollen liver, tense and dark with blood, of the turgid mesenteric veins and injected coils of intestine, shows clearly that an important part of the blood of the whole body has been temporarily arrested in the splanchnic area. How far such an effect enters into the production of "histamine-shock" we shall consider in a later paper. For the present we are concerned with the rapidly evanescent depressor effect of small doses, which cannot be explained on these lines. The plethysmographic records, with which we deal further in a later section, furnish clear evidence against Mautner and Pick's view; but we may first note two other directions in which decisive evidence against it may be found.

*Time relations of the depressor action on injection into different parts of the circulation.* If Mautner and Pick's assumption were correct the latency of the depressor action in the cat and dog should be shortest when the injection is made into the portal vein. We compared the latencies of the action of small doses of histamine with injections into the lumen of the portal vein by a needle inserted through the splenic vein, into the aortic arch by a needle inserted through the central stump of the tied carotid artery, and into the inferior vena cava by a needle inserted through a renal vein. The injection was signalled by a sharp compression of the thick-walled rubber tube connecting the arterial cannula with the manometer, the necessity of accurate alignment of a separate signal-record being thus obviated. Fig. 2 shows a record from such an experiment. It is obvious, even without measurement, that the latency is least with arterial, intermediate with caval and longest with portal injection. It will also be obvious that, though the same dose is given in each case, the fall of pressure produced by intracaval injection is conspicuously deeper than that following intraportal injection, presumably because part of the histamine given by the latter route is destroyed in the liver before reaching the general circulation. The actual duration of the latency of the effect of an injection will necessarily vary with the rate of the blood-flow, so that the effect of an intra-arterial injection given when the arterial pressure is high will appear more promptly than that of an injection given when the pressure is lower; but even when conditions of flow are practically constant, the effect is consistently later in appearance with intraportal than with intracaval injection. Histamine, therefore, produces its depressor action at some point reached earliest by blood in the arteries, later by blood in the systemic veins, and later still by blood in the portal vein. The action cannot, therefore, be located in the liver or the lungs.

*Action on the eviscerated animal.* Further evidence against the view that events in the liver play an important part in the depressor action may be found in the fact that it is produced, without noteworthy impairment, after the vessels to the stomach, intestine and liver have all been ligatured and the whole of the abdominal portion of the alimentary canal has been removed. Fig. 3 shows the fall of blood-pressure produced

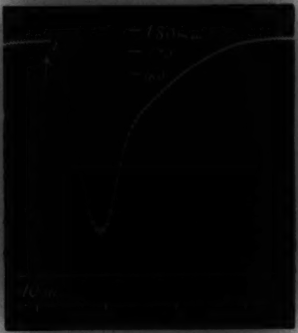


Fig. 3. Effect of 0·025 mgm. histamine on blood-pressure of eviscerated cat.

by 0·025 mgm. of histamine in an animal thus eviscerated, and with the liver therefore excluded from the circulation.

### III. PLETHYSMOGRAPHIC RECORDS FROM THE ANIMAL WITH NORMAL CIRCULATION.

Since **Mautner** and **Pick's** suggestion failed to afford an explanation of the anomalous vasodilator action, we returned to a more detailed investigation of the effect as demonstrated by plethysmographic records, with special reference to hitherto unexplored possibilities of its dependence on the integrity of the nervous connexions of the blood vessels. Such possibilities, as will be seen, were soon excluded, but in the process of their consideration we had taken the opportunity of examining the nature of the vasodilator action of other substances. We ultimately selected two for special study—adrenaline, because in the failure to demonstrate its vasodilator action by perfusion and in the limitation of this action to certain species (it has only been observed hitherto in the carnivora) it presents some points of resemblance to histamine; and acetyl-choline, because it has a more typical vasodilator action, demonstrable even by perfusion of an organ with cold Ringer's solution (**Dale**(7), **Hunt**(8)), occurring in all species on which the observation has been made, and so rapidly evanescent that small doses can be given repeatedly without permanently affecting the condition and responsiveness of the animal. The comparison of the effects of these substances with those of histamine under varying conditions yielded some highly suggestive results, the significance of which could be further tested by experiments on artificially perfused organs.

Many of the experiments now to be described deal with a comparison of effects in the two hind limbs. Care was taken in such cases to insert equal parts of the two limbs into the plethysmographs. The two recording bellows were of identical dimensions, but it unfortunately happened that one was covered with a slightly thicker membrane, so that its movement was rather less prompt and delicate than that of the other. In comparative records the more sensitive of the two bellows always writes the upper of the two records, except when the contrary is stated, and small differences in the direction of greater rapidity and extent of movement recorded in the upper line must not be regarded as significant. It is only to major and unmistakeable differences in this direction that we attribute importance in our argument.

(i) *Direct nature of the vasodilator effects.* We must first exclude the possibility that the action of histamine, and of the other vasodilator substances with which we deal, may be due to an action on the central nervous system or on some more peripherally placed nervous mechanism. The participation of the sympathetic nerve supply in the vasodilator action of histamine had already been excluded by Dale and Laidlaw, but they had not dealt with the possibility that the vasodilator effect might be due to antidromic impulses in sensory fibres, whether excited by stimulation of some part of the central nervous system, or by an axon reflex from sensory nerve-endings, of the type invoked by Bruce in explanation of the local vasodilatation produced by irritant substances. In the case of adrenaline, Hartmann and Fraser(6) as the result of experiments on the immediate effects of nerve-section and on the separate perfusion of an organ while adrenaline was injected into the general circulation, concluded that the vasodilator action was due to an action on the central nervous system. A study of their experiments suggested to us that their results were in part attributable to the use of too large doses, so that some of the dilator effects which they studied were depressor reflexes secondary to the primary pressor effects, and in part to a failure to appreciate fully the immediate effect of nerve section, in producing practically maximal vasodilatation in the affected limb for the time being. Since our own experiments had convinced us that these suspicions were correct, Gruber(11) has published experiments which satisfactorily demonstrate the peripheral nature of the vasodilator action of adrenaline on the vessels of skeletal muscles, by showing that it survives degeneration of the nerves to a leg, though it may be temporarily obscured by the immediate effect of their section. The fact that the dilator action of acetyl-choline can be demonstrated on an isolated organ perfused with a saline solution (Dale, Hunt) had established the fact that this substance produced its effect by peripheral action. There remained a possibility, though perhaps no great likelihood, that this action might be dependent on the integrity of peripheral endings of sensory nerves. Hunt apparently regards the effect of atropine, which abolishes the vasodilator action of acetyl-choline but leaves that of sensory nerve stimulation intact, as strong evidence against this suggestion.

All such possibilities are at once definitely set aside by the reaction to these substances of a limb completely denervated by degeneration of all its nerves. Fig. 4 shows the expansion of a cat's leg, 16 days after section of the sciatic and anterior crural nerves, in response to small intravenous doses of histamine, adrenaline and acetyl-choline, with the concomitant falls of arterial blood-pressure. It may be noted that, in the case of adrenaline only, the expansion is followed by a distinct shrinkage of the limb. Such a result makes it certain that all three substances can produce vasodilatation by a direct action on the blood vessels, which is independent of the persistence of any nervous connexion; a further possibility of explaining the failure of histamine to dilate the vessels of the artificially perfused organ is thereby removed. An apparent elimination of any of these effects, as the immediate result of cutting the nerves to the organ under examination, as in the earlier experiments of Gruber(12) and those of Hartmann and Fraser on adrenaline, must therefore find its explanation along other lines. Nevertheless a comparison of such immediate effects of section on the vasodilator responses to the three substances has yielded results which throw a good deal of light on our problem. We have also to consider whether the direct action, as shown by the denervated organ, accounts for the whole of the vasodilator action of these substances—whether the dilator effect is weakened, unaltered, or enhanced by degeneration of the nerves.

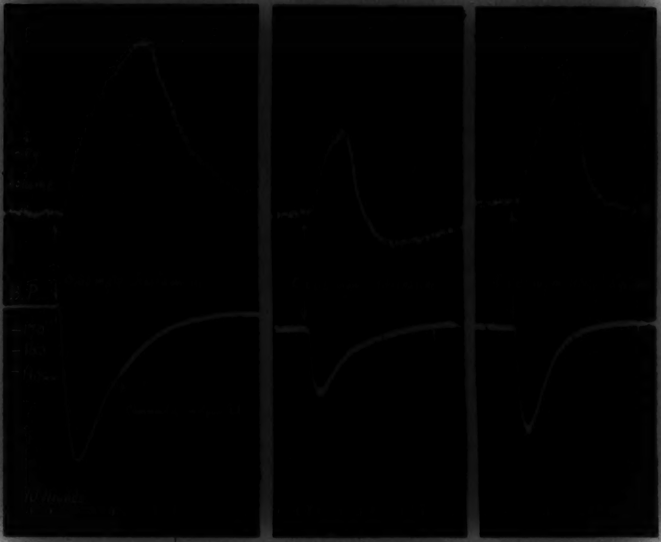


Fig. 4. Volume of leg completely denervated by degeneration; blood-pressure. Intravenous injections as indicated.

(ii) *Volume-changes in the normally innervated leg.* We must note, in the first place, that the hind leg, which is the organ on which nearly all our plethysmographic observations were made, shows remarkable irregularity in the changes of volume which accompany the general fall of blood-pressure produced by small doses of these substances, and in particular of histamine and adrenaline. **Hunt** describes the expansion of the hind limb with a small dose of acetyl-choline as likewise irregular, in comparison with that of the fore limb, but in our experience with very small doses of this substance (0·001 to 0·01 mgm.) the hind leg has very consistently shown a well-defined expansion, corresponding fairly closely with the fall of blood-pressure, the only complication being a slight preliminary shrinkage, which we regard as the passive effect of the dilatation starting earlier in organs nearer the heart. Our experiments on this point have all been made on the cat, and in this species expansion seems to be the characteristic reaction of both fore and hind limbs to acetyl-choline. With histamine and depressor doses of adrenaline, on the other hand, the hind leg with normal nervous connexions shows any kind of volume response from pure expansion to pure constriction. It may be noted, in passing, that evidence of the same variability in the volume change of the limbs may be found in a number of publications dealing with the action of the "depressor substances" found in various organ extracts and products of protein hydrolysis, of which, as **Dale** and **Laidlaw** pointed out, the action on the blood vessels belongs to the same general type as that of histamine. Some authors have regarded such differences as due to specific activities of the extracts from different organs on particular parts of the vascular system(13). Thus one extract will be described as causing vasodilatation in the intestine but constriction in the leg, while another is reputed to cause a greater dilatation in the leg than in the intestine. In the case of some organ-extracts the effect is probably complicated by admixture of pressor with the predominant depressor substances; but the fact that we observe quite as wide a variation in the volume changes of the leg in a series of cats as a result of injecting into each the same dose (0·01–0·02 mgm.) of the pure substance histamine, makes it impossible to accept differences of this kind as indicating a specific localisation of the effects of organ-extracts. Between simple expansion and simple shrinkage all varieties of mixed effect can be observed in the legs of different cats with small intravenous doses of histamine—a small preliminary expansion followed by a more pronounced constriction outlasting the fall of blood-pressure, an expansion interrupted midway by a constriction, and so on.

Several of these varieties can be seen in records reproduced in this paper.

The volume change of the normally innervated leg of the cat, accompanying the fall of blood-pressure usually produced in this species under ether anæsthesia by a small dose of adrenaline (0·001–0·005 mgm.), not only shows the same range of variation as that produced by small doses of histamine, but in practically every instance the particular form of the plethysmogram produced by the leg of a particular cat in response to histamine is reproduced with suggestive fidelity in response to adrenaline. Cf. Figs. 5, 9 (left leg), and 11 (left leg). Such difference as can be detected between the volume changes in the leg of any one cat in response to these two substances is practically always in one direction—a greater prominence of the constrictor phase of the action in the case of adrenaline than in the case of histamine.

This tendency of the constrictor action to greater relative prominence with adrenaline gives a clear hint as to its nature. It cannot be due to the excitation of the vasomotor centre, which is stated to follow a fall of general arterial pressure as a result of bulbar anæmia (cf. Hill(14)), since, if it were so, it should be most prominent in the case of histamine and acetyl-choline, which in the doses usually employed by us cause a much larger fall of arterial pressure than the greatest obtainable with adrenaline; nor is it possible to attribute to such secondary action of the vasomotor centre a constrictor action which sometimes begins as soon as the fall of pressure. In the same way it cannot be mainly a mere passive effect of the general fall of pressure, for in this case again it should be proportionate to the fall produced, and should not outlast the depressor effect, as it frequently does. The nature of this constrictor phase of the action of these two substances, and the consequent complexity and variability of the plethysmograms given especially by the hind leg in response to them, becomes clearer when it is remembered that each of them possesses not only the vasodilator action which is the subject of our immediate study, but a vasoconstrictor action. This, in the case of adrenaline, is the more obvious and characteristic effect, becoming generally predominant when the dosage is but slightly raised beyond about 0·005 mgm., which is the maximum dose with which, in the average cat under ether, a purely depressor effect with adrenaline is to be expected. Any untoward influence, such as undue prolongation of the experiment, even though attended with but a small decline of level of the arterial pressure, usually results in the replacement of the depressor by a pressor action of adrenaline even in this lower range of dosage. In the case of histamine the general vasodilator fall of pressure is a more stable and characteristic effect of small doses, and the fact that these also possess a vasoconstrictor action only becomes distinct under conditions of artificial perfusion; but experiments of the latter kind leave no doubt as to its existence. Acetyl-choline, on the other hand, appears to have a purely vasodilator action in the low range of dosage which we have used throughout these experiments. The vasoconstrictor effects of relatively very large doses have been discussed elsewhere by Dale and by Hunt and do not concern us here. A primary difference between the effects of these substances in the doses with which we are dealing is, therefore, that with either histamine or adrenaline we have a complex of opposed actions, a vasodilator and a vasoconstrictor effect, either of which may in a particular case be predominant in an organ such as the leg, whereas small doses of acetyl-choline have a purely vasodilator action. The relative uncertainty of the volume-effect with histamine and adrenaline and constancy of that with acetyl-choline find in this difference a sufficient explanation. The only point needing additional emphasis at this stage is the aforementioned parallelism between the effects of histamine and adrenaline on the leg volume in different experiments. In both cases we now know that both types of effect, dilator and constrictor, are due to direct and peripheral action; so that the parallelism between the effects of the two substances can only be interpreted as indicating that the conditions of vascular tone which favour the predominance of either vasoconstriction or vasodilatation in the normally innervated leg are the same for histamine as for adrenaline, while the vasodilator action of acetyl-choline is relatively independent of such conditions.

The explanation which immediately suggests itself is that which Cannon and Lyman(15) gave for the variations in the effect of small doses of adrenaline on the cat's blood-pressure, namely, that the condition determining the response in the direction of vasodilatation or vasoconstriction is simply the state of arterial tone at the time of the experiment. On this view, when the tone of arteries in the leg is high histamine or adrenaline should cause them to relax, when it is low to constrict, so that in either case the tone approaches what Cannon and Lyman call a "critical level." The sufficiency of this simple conception can be tested by observing how the volume-response of the leg to these substances is affected by changes in arterial tone deliberately introduced. So long as it leaves unimpaired the vitality and sensitiveness of arterial muscle, any influence which produces relaxation of the arteries in the leg should tend to promotion of the constrictor and elimination of the

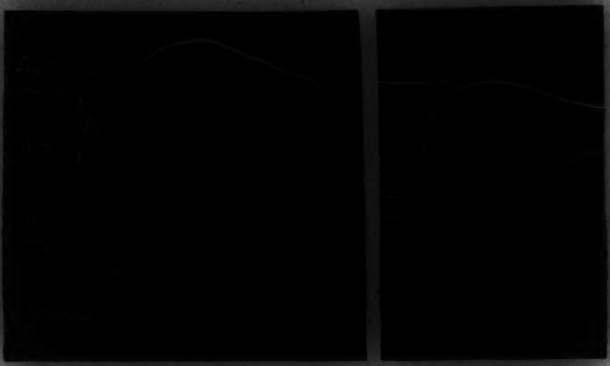


Fig. 5. Volume of normal leg and blood-pressure. At *A* 0·02 mgm. histamine; at *B* 0·004 mgm. adrenaline.

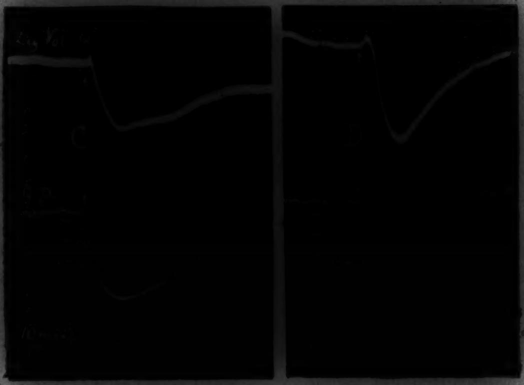


Fig. 6. Continuation of experiment shown in Fig. 5. Sciatic and anterior crural nerves have now been cut. At *C* and *D* 0·02 mgm. of histamine and 0·004 mgm. of adrenaline, as before.

dilator action of both these substances. We shall see that under this test the explanation fails.

(iii) *Immediate effects of nerve section.* When the sciatic and anterior crural nerves are cut the immediate effect is a dilatation of the arteries of the leg, which, as **Bayliss**(16) has shown, is not adequately explained by the interruption of the flow of impulses from the vasomotor centre along sympathetic fibres, but is due in part to the excitation of sensory vasodilators by the mechanical stimulus of the section. The dilatation is immediately made obvious in the plethysmogram by the large increase in the amplitude of the volume-pulse. The persistence of the effect is variable, but the excess of volume-pulse in the decentralised limb usually remains obvious throughout the further course of an ordinary experiment.

In cases in which histamine and adrenaline cause a dilatation of the limb while the nerves are intact, it sometimes happens that the same doses, injected again immediately after the nerves are cut, cause a simple shrinkage of the limb, which may have the appearance of passively following the fall of arterial pressure. Such a change of response is illustrated in Figs. 5 and 6 which further illustrate what was said above concerning the greater relative prominence of the constrictor element in the action of adrenaline. **Hartmann** and **Fraser's** contention that the vasodilator action of adrenaline is central in origin was partly based on observations of this kind. This, however, is by no means a constant or even the most frequent and characteristic type of change in response to these substances produced by section of the nerves. Figs 5 and 6, indeed, are taken from the only experiment, among many which we made, in which the change in this direction was thus definite. Fig. 7 shows an entirely different type of change, the practically pure constrictor response of the normal (left) leg to 0·01 mgm. of histamine being replaced by simple dilatation in the case of the other (right) leg, the nerves to which have

Fig. 7. Effect of 0·01 mgm. histamine. Volumes of leg with nerves freshly cut and of normal leg; blood-pressure.

been cut a few minutes previously. Note the greatly exaggerated volume-pulse in the right leg, indicating that the arterial dilatation produced by nerve section still persists. It may be added, as of incidental interest, that the cat giving this record showed a very high degree of sensitiveness to histamine. Tests for the lower limit of effective dosage showed a distinct fall of arterial blood-pressure and definite expansion of the right (decentralised) leg with 0·000,000,01 mgm., but no effect with one-tenth of this dose. The limit was therefore the same as that found by Hunt for acetyl-choline. Such extreme sensitiveness to histamine is, however, not usual. Fig. 8 is taken from the record of an experiment on a cat in which the nerves to the left leg had been cut 26 days previously. At the beginning of the experiment injections of small doses of adrenaline and histamine (in each case 0·01 mgm.) caused dilatation of the left (denervated) and constriction of the right (normally innervated) leg. We shall see later that this readier response of the denervated leg to the dilator action is the general rule. In the middle of the experiment the nerves to the right leg were cut, and the same doses of adrenaline and histamine repeated. Although the arteries become somewhat dilated in consequence, as shown by the increased volume-pulse, 0·001 mgm. of adrenaline, at *A*, now causes distinct expansion of the right leg, subjected to recent nerve section, while the completely denervated left leg now constricts. This constriction of the denervated limb is a very uncommon result in our experience, and it is the more interesting, therefore, to

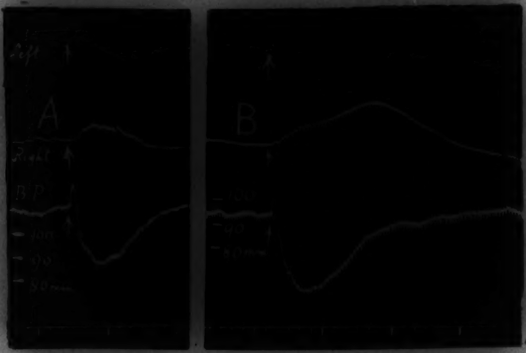


Fig. 8. Left leg completely denervated (unusual constrictor response); nerves to right leg freshly cut. At *A* 0·001 mgm. adrenaline; at *B* 0·01 mgm. histamine.

observe that the same reversal of reaction has occurred during the experiment in the case of histamine, which now (at *B*) similarly causes shrinkage of the completely denervated left leg, expansion of the recently decentralised right.

In a series of experiments on the immediate changes in response due to nerve section this correspondence between the effect of histamine and adrenaline is clearly traceable. It can be said with certainty that if a small dose of histamine (of the order of 0·01 mgm.) causes constriction of a limb, adrenaline in similar low dosage also causes constriction; if adrenaline causes dilatation histamine causes dilatation. The converse statements do not always hold good, for, as already stated, the dilator effect of adrenaline is more liable to be overpowered by its constrictor effect, so that cases may be found in which histamine causes dilatation while adrenaline causes constriction. But the broad agreement is sufficiently striking to warrant the suggestion that there is some condition of the limb vessels which favours in common the vasodilator effects of histamine and of adrenaline, and that this condition is not the state of tone of the arteries. We shall have to consider also the effect of changes in arterial tone on the vasoconstrictor action, which complicates the vasodilator action even of small doses of histamine and adrenaline, and in the case of the latter becomes the predominant effect when the dose is raised above a certain low level. Before doing so, however, it will be convenient to examine the immediate effect of nerve section on the response to acetyl-choline, which has apparently an unmixed vasodilator action in the doses with which we are concerned. We have made fewer such experiments with this substance than with the others, but the results have been uniform and have shown a very significant divergence from those obtained with histamine and adrenaline, when the changes of response to all three were recorded in the same experiment. When the nerves to the leg in the plethysmograph were cut, and the arteries in consequence became dilated, the expansion shown by the leg in response to a small dose (*e.g.* 0·001 mgm.) of acetyl-choline has always been conspicuously less than that shown by the same leg while its nerves were intact, even when the effect of the nerve section was so to increase the dilator action of histamine and adrenaline that a constrictor action of these substances on the normal limb was replaced by a dilator action when the nerves were cut. In the experiment of which Fig. 9 reproduces part of the record, both limbs had constricted while their nerves were intact in response to small doses of histamine (0·01 mgm.) and adrenaline (0·004 mgm.). Under the same condition the two limbs had shown approximately equal expansions in response to acetyl-choline (0·001 mgm.). The nerves (sciatic and anterior crural) of the right leg were then cut, and the doses repeated, with the results shown in Fig. 9. It will be seen that the intact left leg still shrinks in response to histamine and adrenaline, but dilates well with acetyl-choline. The effect of nerve section on the right leg, the arterial dilatation in which is, as usual, visible in the large volume-pulse, is to cause this limb to show a small, but quite definite expansion with adrenaline and histamine, whereas its expansion with acetyl-choline is very small in comparison with that of the normal leg. Fig. 10 shows a similar comparison of the volume effects of histamine and acetyl-choline on a normal (left) leg and freshly decentralised (right) leg. Again it will be seen that the cutting of the nerves, with its resultant arterial dilatation, has caused an increase in the dilating action of histamine, a decrease in that of acetyl-choline.

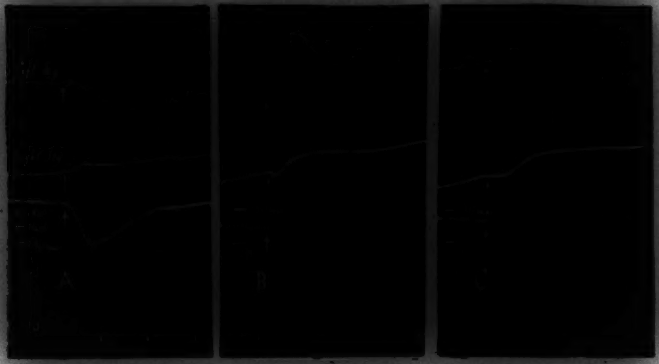


Fig. 9. Left leg normal, nerves to right leg freshly cut. At *A*, 0·004 mgm. adrenaline; at *B*, 0·001 mgm. acetyl-choline; at *C*, 0·01 mgm. histamine.

Such a result, considered by itself, might be taken to indicate that the vasodilator action of acetyl-choline was partly produced through action on nerve centres. This interpretation is excluded, however, by the effect of allowing the nerves to degenerate completely after section, when, as will be described below, the dilatation in response to acetyl-choline becomes greater than that of the normal limb. The diminished reaction immediately following nerve section must, therefore, be related to the change in tone of the limb vessels which is the immediate consequence of the operation. The facts are entirely compatible with the view that the dilator action of acetyl-choline is due to inhibition of the tone of arterial muscle; when this tone is already relaxed as the result of the vasodilator stimulus of nerve section acetyl-choline produces a smaller dilatation.

It is beyond doubt, also, that the vasoconstrictor actions of histamine and adrenaline are, at any rate, largely effects on arterial muscle. That contraction of isolated strips, from arteries of different parts of the system, is produced by minute doses of both these substances has been shown by numerous workers on their action. Later in this paper we shall produce evidence of the constriction produced by histamine on a purely arterial system perfused artificially. We should expect, accordingly, that relaxation of the arteries of an organ as caused by recent section of the nerves would intensify the constrictor action of these substances by giving a wider range for its action. The point is obviously difficult of demonstration by ordinary plethysmographic methods, for, as we have seen, the vasodilator action of very small doses of these

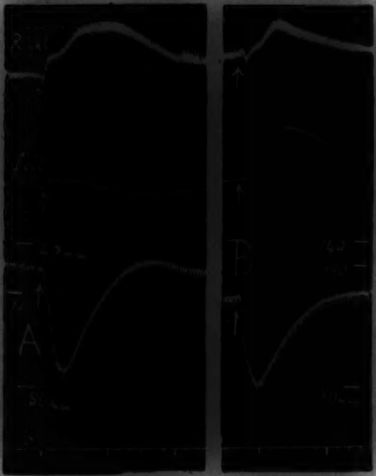


Fig. 10. Left leg normal, nerves to right leg freshly cut. At *A*, 0·01 mgm. histamine; at *B*, 0·001 mgm. acetyl-choline.

substances is more often intensified than impaired by such nerve section, so that the impression is often obtained that the constrictor effect has been weakened by the arterial dilatation following nerve section. This impression can usually be corrected by pushing the dose higher, when the superiority of the constrictor effect also in the decentralised limb becomes obvious.

Occasionally, in common with others who have worked on the subject, we have come across a cat in which even the smallest doses of adrenaline produced practically no vasodilatation, though histamine acted as usual; and in such cases we have observed an intensification of the constrictor action of small doses of adrenaline as the result of nerve section, though the dilator effect of histamine was increased by the same procedure. So far, then, as the complexity of the response to histamine and adrenaline allows us to determine it, it would appear that, in the constrictor effect of both these substances, as in the dilator effect of acetyl-choline, we have probably an action on arterial muscle, varying in the range of its effect with the state of tone of the arteries. In the case of adrenaline this is, of course, the obvious and prevalent conception of its vasoconstrictor effect. We have seen, on the other hand, that their dilator effect varies in a manner having no apparent relation to arterial tone and the question necessarily arises whether there is any other structure in the vascular mechanism, capable of maintaining a tone which, like that of the arteries, relaxes in response to an appropriate stimulus, and which varies independently of the tone of the arterial muscle. The suggestion almost inevitably presents itself, that the dilator effect may be due to action on the walls of the capillaries. We may leave it as a mere suggestion at present, until we have considered evidence of other kinds.

(iv) *Effect of complete denervation.* We have already seen that the leg to which the nerves have been allowed to degenerate completely after section gives a full dilator response to small doses of all the three substances with which we are dealing. We have now to add that this dilator effect is in all three cases habitually, though not quite invariably, much larger than that shown by the corresponding normally innervated limb. Fig. 8 shows an exceptional case of constrictor reaction to small doses of both adrenaline ($A$) and histamine ($B$) after complete denervation (left leg). There is, indeed, a general increase of responsiveness to chemical stimuli of all kinds; thus in one case the denervated limb showed a much larger dilatation than the normal, not only with the three substances specially under consideration, but with amyl nitrite and caffeine as well. Such a sensitized limb further showed a much more pronounced constriction than the normal with a pressor dose (*e.g.* 0·05 mgm.) of adrenaline. Possibly this concomitant increase of sensitiveness to vasoconstrictor effects affords an explanation of the rare cases in which we observed that a moderate dose of histamine (0·02 mgm.) caused smaller expansion of a denervated limb than of the corresponding normal, which happened in such cases to expand unusually well.

The experiments on the denervated leg with acetyl-choline and the other more normal vasodilator substances, such as amyl nitrite, have only been made hitherto after an interval sufficient to allow complete degeneration (14 days and upwards after section). With histamine, on the other hand, and in a few cases with adrenaline also, we have seen that the improved dilator response appears almost immediately after the nerves have been divided, at a time when the dilator response to acetyl-choline is weakened by the slackness of the arterial tone. We have observed the exaggerated dilator response to histamine at all periods from 15 minutes to 2 months after nerve section. It occurred to us that observation of the state of circulation in the denervated as contrasted with that in the normal leg might throw further light on the mode of action.

The flushing of a limb as the immediate result of section of its nerves has been known since the early experiments of Bernard and of Schiff. There is no clear and consistent account, however, of the relation of the temperature of the limb to the state of tone of its superficial vessels, as judged by inspection, during and after degeneration of the nerves following section. Goltz(17) observed that the dog's limb with cut nerves remained warmer than the normal limb for varying periods up to four weeks after the operation. Luchsinger(18), using cats with unpigmented feet, described the pads of the operated foot as redder than those of the normal for 3 to 4 days after section, and thereafter as of equal redness at the ordinary temperature, though at any period the normal pads became the redder if the animal was warmed. We have observed a persistent and curious form of contrast which does not seem to have been noticed previously. We also used cats with unpigmented feet. In our experience the flushing which follows immediately on section of the sciatic and anterior crural nerves passes off rapidly. Often by the time the small operation wound has been sewn up, and while the animal is fully under the influence of the anæsthetic, the operated foot has become distinctly paler than the normal control. On the following day, and for the rest of the period of observation (up to 2 months), the contrast presented by the two feet is striking and suggestive, the pads of the denervated leg being as a rule conspicuously *paler* than the normal, and at the same time very noticeably and surprisingly *warmer* to the touch. The contrast is especially conspicuous in cold weather, under which conditions the pads of the normal hind foot are cold to the touch, though they usually present a bright, slightly bluish pink colour. In cases in which this contrast between warm pallor and cold congestion has been well marked, we have invariably observed a conspicuously superior dilator response to histamine on the part of the denervated leg, often contrasting with a constriction of the normal. Fig. 11 shows the response of a leg two days after section of the nerves, and of the corresponding normal leg, to 0·001 mgm. doses of histamine (*A*) and adrenaline (*B*). The similarity of the two effects is clearly shown. Fig. 12 shows the reaction to 0·01 mgm. of histamine 5 days after nerve section, in a cat in which this contrast of temperature and colour was consistently well marked.

Fig. 11. Right sciatic and anterior crural nerves cut 2 days previously; left leg normal. At *A*, 0·001 mgm. histamine; at *B*, 0·001 mgm. adrenaline.

Fig. 12. Right sciatic and anterior crural nerves cut 5 days previously. Left leg normal. Effect of 0·01 mgm. histamine.

It seemed highly improbable that the difference in surface temperature was due to defect of evaporative cooling on the operated side, owing to the paralysis of the sweat glands on the pads, and a simple experiment established the reality of the difference and its independence of sweating. We compared the rates at which the two paws raised the temperature of equal quantities of cold water, in which they were immersed. The cat used was that which furnished the record reproduced in Fig. 12. Into each of two small glass cylinders, fitting easily over the paws, 10 c.c. of water, at room temperature, were poured. The paws were immersed to equal depths and a thermometer was inserted into the water alongside each. Slight up-and-down movements of the cylinders kept the water well mixed. The following figures show the rates at which the temperature rose in the two cases:

| Time after immersion in seconds | Temperature of water | |
|---|---|---|
| | Denervated paw | Normal paw |
| 0 | 11 | 11 |
| 45 | 15 | 13·75 |
| 60 | 17 | 14 |
| 90 | 18 | 14·5 |
| 120 | 18·5 | 15 |
| 150 | 18·5 | 15·5 |
| 180 | 18·5 | 16 |
| 210 | 19 | 17 |
| 230 | 19·5 | 17·5 |
| 250 | 19 | 18 |
| 280 | 19 | 18·5 |
| 300 | 19 | 19 |
| 345 | 20 | 19 |
| 405 | 21 | 20 |
| 450 | 22 | 20·5 |

It will be seen that the greatest difference in rate of warming occurs soon after immersion, the maximum difference between the two temperatures being reached at 1½ minutes. Such a difference in the rates at which the paws give up heat to water surrounding them can only have one explanation. It is not credible that the blood reaching the skin by the arteries in the two cases differs significantly in temperature. The only reasonable explanation is that the blood is circulating more rapidly through the denervated paw than through the normal, and this greater rapidity of circulation, associated with surface-pallor, can only be attributed to the capillary area being more restricted in relation to the volume of blood reaching it by the arteries. In other words, the relative pallor and warmth of the denervated paw must be due, not to constriction of the arteries supplying it, but to a condition of tone of the capillaries, such that the blood courses through them in a restricted but rapid current; the relative flush and coldness of the normal paw must be due not to a more dilated condition of its arteries, but to such relative width of its capillaries that the blood spreads out in them into a broad, sluggish stream. The difference may be compared with that seen between the hands of different persons in cold weather. In one, with good tone and reactivity of the capillaries, the skin of the hands is pale but warm; in another, in whom the capillaries are atonic and poorly reactive, the hands are dusky red, congested and unpleasantly cold to the touch, owing to the sluggish circulation through the dilated capillaries.

It is of interest to note that a smaller difference in surface temperature can often be observed between the fore-paws and hind-paws of a cat in cold weather, the fore-paws being distinctly warmer to the touch. In such cases we have observed a similar contrast in the reactions to histamine, the fore-limb dilating with a dose which causes constriction of the hind-limb (Fig. 13).

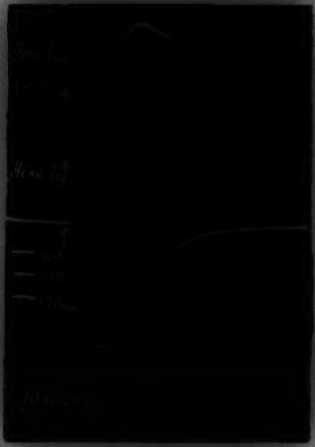


Fig. 13. Effect of 0·04 mgm. histamine on volumes of normal fore and hind legs and blood-pressure.

If observation were limited to the condition after complete degeneration, the association of this condition, which we regard as indicating intensified reactivity of the capillaries, with enhancement of the dilator reaction to histamine and adrenaline, could not be regarded as of great significance; for at this stage the reaction is also abnormally sensitive to acetyl-choline, amyl nitrite, etc., the action of which on arterial muscle is not to be doubted. We have shown, however, in dealing with the immediate results of nerve section, that there is frequently recognisable a stage at which, though the arterial dilatation resulting from nerve section still persists, the dilator reaction to histamine and adrenaline is already intensified while that to acetyl-choline is diminished. It was of interest, therefore, to observe the state of capillary tone at this stage. The following experimental record deals with observations made without the aid of the plethysmograph, the unpigmented pads of the hind feet being merely inspected.

In a large male cat, with unpigmented feet, the sciatic and anterior crural nerves on the right side were cut aseptically under ether anæsthesia. As soon as the small wounds had been stitched and dressed the hind feet were inspected. The pads on the right (operated) side were found to be distinctly redder than those on the left. The right pads were also slightly the warmer to the touch, and were sweating profusely. They rapidly grew paler and the sweating ceased. Five minutes later the right pads were conspicuously paler than the normal left pads, but now felt definitely warmer. They were watched a few minutes longer, to make sure that this contrast had become stable. Intravenous injections of small doses of histamine, acetyl-choline and adrenaline were then given, the cat being still under the influence of ether. After each injection the stable contrast was re-established before the next was given, so that at the time of giving each injection the right pads were paler than the left.

Histamine 0·01 mgm. Right pads flush, while left become slightly paler. While the flush on the right side is passing off a weaker flush on the left side appears, outlasting the more pronounced effect on the right. Both sides then became paler, and the original contrast is slowly restored and becomes stable.

Acetyl-choline 0·001 mgm. Right pads show no definite change of colour; left pads show a well-marked flush, the contrast with the right being decidedly intensified. This passes off and the original condition is restored.

These doses were then repeated, and another observer, unaware of the order in which the drugs were being injected, reported the same effects.

Adrenaline 0·001 mgm. After a longer latent period than that seen with the other two, the right pads show a pronounced flush, while the left become paler so that the right become decidedly the redder. As the effect on the right pads subsides a weaker secondary flush is seen on the left side. This passes off leaving both sides pale, and the original contrast is then slowly re-established.

It will be seen that, in this case, the condition of pallor and relative warmth has already appeared as the result of nerve section, and histamine and adrenaline already show an enhanced dilator effect on the operated side, at a period 10 to 20 minutes after the nerves had been cut. The uniform result of our plethysmographic records has been to show that at this period the arteries are still dilated, the volume-pulse being much increased on the operated side. Whenever it has been tried the dilator effect of acetyl-choline has been diminished at the corresponding stage. Similarly in these observations acetyl-choline produced no perceptible reddening of the pads on the operated side, though it caused distinct flushing of the normal pads.

For our present purpose the important points are that the condition of relative pallor and warmth may appear very early after nerve section, and with it an exaggerated dilator response to small doses of histamine and adrenaline. at a stage when the dilator response to acetyl-choline is depressed. In so far then as we are correct in interpreting the warm pallor as indicative of capillary tone, the suggestion is strengthened that histamine and adrenaline exert their dilator action on capillary walls, while acetyl-choline dilates arterioles.

One recent experiment should be mentioned, in which the nerves of the leg were thoroughly cocainised before section. In this instance the dilator action of acetyl-choline also was increased as the immediate result of the nerve-section. It seems clear, then, that the loss of arterial tone which normally reduces the effect of acetyl-choline at this stage is due to stimulation of vasodilator fibres, and not merely to loss of constrictor tone.

(v) *Effects of prolonged anæmia and cooling.* In searching for an explanation of the failure of histamine to exhibit its vasodilator action on the artificially perfused vessels of a surviving organ, we considered the possibility that the interval between cessation of the normal and commencement of the artificial circulation might play a part. We shall later show reason for believing this suggestion to be incorrect, but it led us to test the influence on the response to histamine of temporary anæmia produced by clamping the arteries to an organ in the living animal. Later we made comparative tests of the alteration in the reaction to acetyl-choline produced by the same procedure. It proved impracticable to test the effect of such treatment on the vasodilator effect of adrenaline. We have already drawn attention to the relative impermanence of this effect, owing to the fact that it readily becomes overpowered by the vasoconstrictor action, even with small doses. In several attempts to determine the effect of temporary anæmia we observed, indeed, that a vasodilator was replaced by a vasoconstrictor effect of adrenaline in the organ under experiment; since, however, the action on the general arterial pressure had simultaneously changed from a depressor to a pressor effect, the local result could not be regarded as significant. We confined our attention, therefore, to the changes in the action of histamine and acetyl-choline under these conditions. A few experiments were made on normally innervated legs showing a good dilatation with histamine, but for most of these experiments on the effects of temporary anæmia we chose cats in which the nerves to one hind leg had been allowed to degenerate after section. We had, therefore, an organ which could be depended on to give initially a full dilator response to both histamine and acetyl-choline. Precautions were necessary to guard against an anastomotic supply of blood to the limb while the vessels were occluded, so that a real ischæmia could be established.

For this purpose all branches from the lower end of the aorta and vena cava to the lumbar muscles, etc., were tied up to about 1 cm. above the origin of the inferior mesenteric artery. The latter was also tied, cut, and the lower end of the rectum double ligatured and cut through. In female cats the vagina and the vessels accompanying it were similarly double ligatured and divided and the ovarian vessels tied off. The aorta was tied between the origin of the external iliac arteries and its terminal bifurcation into the internal iliacs, which have this separate origin in the cat. The muscles and skin of the back and abdominal wall, at a level just above the origin of the inferior mesenteric artery, were then mass ligatured in three sections, the strong string ligatures being passed by transfixion.

The external iliac artery and vein on the side of the normal leg were clamped close to their junction with aorta and vena cava and ligatured about a centimetre lower down. A cannula was inserted into each between ligature and clamp. Through these the vessels of the denervated limb could be washed out when the aorta and vena cava were clamped. Cannulæ for recording blood-pressure and making injections having been tied into a carotid artery and jugular vein, a plethysmograph was applied to the denervated leg, which was to be the subject of observation, and the record started. Preliminary injections of histamine and acetyl-choline were first given. The vasodilator effects of two substances on such a denervated limb give curves of very similar contour, but when doses are chosen which cause about equal falls of general arterial pressure the expansion of the leg caused by histamine is as a rule distinctly the greater.

When the vessels to the leg are occluded, even for a brief period, and the circulation then restored by their release, the immediate effect is a wide dilatation of the vessels of the leg, as shown by the great increase in volume of the limb beyond its original size and the very large volume-pulse. **Roy** and **Graham Brown** (19) have shown that this dilatation after temporary anæmia affects not only the tone of the arterioles but of the capillaries also. At this stage, if a record is taken as soon as the volume of the limb has become approximately stable, the fall of arterial pressure caused by injection of either histamine or acetyl-choline is accompanied by a shrinkage of the limb which has all the appearance of a merely passive effect. As the vessels regain tone the dilator effect of acetyl-choline reappears and later that of histamine also. The dilator effect of histamine, however, recovers at a distinctly lower rate than that of acetyl-choline. Nevertheless it eventually reappeared, even after the flow of blood through the leg had been stopped for as long as an hour. The disparity between the rates of recovery of the action of the two substances was apparently accentuated if the anæmia was rendered more complete, and the additional factor of cooling during the anæmic period introduced, by washing the vessels of the leg out with cold saline, as above described, and temporarily replacing the warm water in the plethysmograph with cold. When the normal circulation was again restored a stage of recovery could be found at which acetyl-choline produced a definite dilator action, while a dose of histamine, causing an approximately equal fall of arterial pressure, produced a simple constriction of the limb (Fig. 14). Later, when the reaction to acetyl-choline had still further recovered, histamine again caused dilatation of the limb, but the effect was relatively a small one, whereas, when the same doses of the two substances were given before the period of anæmia and cooling, the dilator effect of histamine was distinctly the greater.

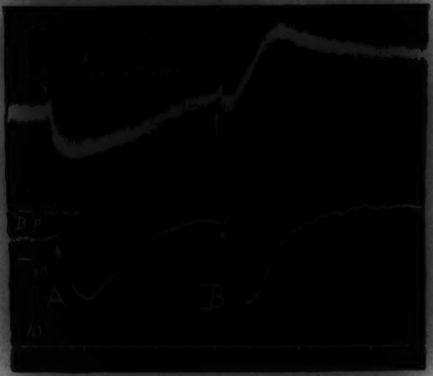


Fig. 14. Volume of leg after anæmia and cooling; blood-pressure. At *A*, 0·01 mgm. histamine; at *B*, 0·001 mgm. acetyl-choline.

Comparing the effects of temporary anæmia with those immediately following nerve section, we have in both cases a long persistent dilatation of the vessels of the limb. This causes, in both instances, a weakening of the dilator effect of acetyl-choline, in accordance with the expectation that an arterial dilator will exhibit relatively little action on arteries already dilated. In the case of histamine, on the other hand, we have the significant contrast, that whereas its dilator effect may be and indeed usually is accentuated during the period of dilatation following nerve section, in that following anæmia its dilator effect is weakened to a greater degree and recovered more slowly than that of acetyl-choline. Again the evidence suggests that the vasodilator action of histamine is produced on some part of the system of blood vessels other than the plain muscle of the arterioles, and the additional conclusion is indicated that the structure in question is one of which the tone is not necessarily weakened by the stimulus of fresh nerve section, but is more seriously and lastingly impaired by anæmia and cooling than that of the arterial muscle. The evidence, so far as it goes, is again consistent with the view that the dilator action of histamine is an effect on the tone of the capillaries; for there is no evidence that this tone is regularly weakened by nerve section like that of the arteries, while there is evidence of its susceptibility to temporary anæmia and cold.

## IV. Distribution of the vasodilator action.

Before we consider the distribution of the effects in the different organs of one animal, there are points of interest to be noted in the manner in which the vasodilator action of the three substances appears in different species. The vasodilator action of acetyl-choline has been observed in all animals on which the experiment has been made. Hunt quotes experiments on rabbits, cats and dogs. We have ourselves observed the effect in a monkey as well. There is no apparent difference in the order of intensity of the effect in the different types.

The vasodilator effect of adrenaline, on the other hand, has hitherto only been described in carnivora. It seems definitely to be absent in the rodents. It is at least highly suggestive that the vasodilator action of histamine shows an analogous specific restriction. In the cat and dog it is the obvious and predominant feature of the effect on the circulation; in these species a vasoconstrictor action is also present, but only becomes obvious in special circumstances. In the rabbit and guinea-pig, on the other hand, Dale and Laidlaw found that the effect of histamine, under conditions excluding complications due to interference with respiration, was a simple rise of blood-pressure due to vasoconstriction. In some recent experiments on the effect of histamine on anæsthetised rats we found no evidence of the depressor, vasodilator action, though this species has provided the hitherto unique example of plain muscle (uterus) of which the tone and rhythm are inhibited by histamine (Guggenheim). It is not known how far the correspondence in distribution between the vasodilator actions of histamine and adrenaline holds good for other species. Dale and Laidlaw observed a very pronounced vasodilator action of histamine in the monkey, and a depressor action, probably of the same type, in the fowl, but the reaction of these types with minute doses of adrenaline has apparently not been recorded. We have not as yet had the opportunity of observing it ourselves. The persistent pressor action of larger doses of adrenaline, observed by Barger and Dale(20) after ergotoxine, does not necessarily show that adrenaline has no vasodilator action in this species, but only that the vasoconstrictor action is not eliminated by ergotoxine. So far, then, the action of all three substances has only been compared in the cat, dog, and rodents; and of these only the cat and dog show vasodilator effects with adrenaline or histamine, while acetyl-choline, like better known vasodilators such as the nitrites, shows similar effects in all. The vasoconstrictor actions of adrenaline and histamine, again, can be detected in carnivora and rodents alike.

In comparing the action of the different substances on different organs and tissues we are less inclined than some writers to attribute importance to quantitative differences, since experience has convinced us how widely the volume-change exhibited by any organ may vary with changes in the tone and responsiveness of its vessels. Certain broad and apparently constant differences of reaction may be noted. The kidney seems peculiarly insensitive to the vasodilator action of all three substances, showing a reduction in volume when other organs expand. Here again, however, a difference may be observed, in that with acetyl-choline the shrinkage seems to be purely a passive effect of the general fall of blood-pressure, while histamine and adrenaline, in doses producing a depression of the same magnitude, cause a shrinkage of the kidney disproportionately great. The circulatory scheme of the kidney, however, with its secondary system of efferent arterioles from the glomeruli, presents complexities which make interpretation of its volume changes peculiarly difficult.

The point probably of most interest is the distribution of the dilator effect between the vessels of the skin and those of the skeletal muscles. Hoskins, Gunning and Berry(21) have drawn attention to the greater regularity and extent of the dilator effect of adrenaline in the bared muscles of a limb than in the whole limb with its normal covering of skin. We have observed a parallel contrast in the case of histamine. When a cat's leg constricted with histamine and with depressor doses of adrenaline, we found, on removing the skin and amputating the foot at the ankle, and replacing the warm water in the plethysmograph with saline at the same temperature, that the bared musculature of the leg showed expansion with the same doses of both substances (Fig. 15). If we can assume that the response of the vessels in the musculature has not been altered by the operation of skinning, it is evident that the preponderant constriction seen with the intact leg must have been an effect on skin vessels. We do not regard this, however, as necessarily indicating a specific difference of reaction between the vessels of the skin and those of the muscles. When the leg has been denervated, by allowing time for degeneration of its nerves after section, there can be no doubt that the skin vessels participate in the large expansion of the whole leg in response to histamine or adrenaline. In unpigmented pads of the feet the vasodilator effect can then be directly observed, and in a leg so sensitized to vasodilator effects we found that removal of the skin merely reduced the extent of the expansion in response to histamine. (The effect of skinning the denervated leg on its response to adrenaline was unfortunately not tested.) We are disposed, therefore, to regard the predominance of constrictor effects frequently seen in the normally innervated limb, and the increased prominence of the dilator effects produced in such a limb by removing the skin, as indicating that the normal vasomotor control, affecting chiefly the skin vessels, tends to produce the conditions of tonus which we have described in an earlier section as favouring the preponderance of the constrictor over the dilator effects of histamine and adrenaline. As we there pointed out, this condition is especially liable to be found in the hind limbs during cold weather, and it is obvious that the vasomotor adjustment to external temperature will affect the vessels of the skin especially.

Fig. 15. Effect of skinning the leg on volume-response to histamine and adrenaline.

*A*—0·02 mgm. histamine } on intact leg.
*B*—0·002 mgm. adrenaline } on intact leg.
*C*—0·02 mgm. histamine } on skinned leg.
*D*—0·004 mgm. adrenaline } on skinned leg.

The meaning of the varying reaction of the skin vessels to histamine and adrenaline, and of the relatively constant dilator response of those of the muscles, becomes clearer in the light of the evidence which we have already given, and which we shall later reinforce by more direct evidence, in support of the suggestion that the vasodilator action of these substances is an effect on capillaries, the antagonistic vasoconstrictor action an effect mainly on arterial muscle. The variable tone of the arterioles of the skin, and its susceptibility to sympathetic control, are familiar and conspicuous, while the effect of nervous impulses on the arterioles to skeletal muscles is obscure and uncertain, being complicated under conditions of normal muscular activity by vasodilator phenomena which are often regarded as effects on capillaries, possibly due to the products of the muscular activity. If histamine and adrenaline also owe their vasodilator effects to action on capillary walls, it might be expected that this would often be obscured in the skin by their stimulant effect on arterial muscle. Especially would this be so in the case of adrenaline, reproducing in the arterioles of the skin the effects of their rich supply of sympathetic vasoconstrictor nerves. In the muscles, on the other hand, a vasodilator effect on capillaries might be expected to be more usually preponderant. The facts are again, therefore, in accordance with the supposition of a capillary-dilator effect. On this supposition we should expect the contrast between the response of skin and muscle to be greater in the case of adrenaline, with its intense arterio-constrictor action, than in that of histamine, with its weaker effect on arterial muscle. In several of our experiments this expectation also has been realised, though the difference is not obvious in Fig. 15.

Hunt mentions and illustrates an experiment in which the volume response of the fore limbs to acetyl-choline was recorded, one being intact and the other skinned. He found that the intact limb showed a much more pronounced expansion than the skinned muscles. Similarly he found that the venous outflow from a cutaneous vein underwent a much greater proportionate increase than that from a muscular vein when acetyl-choline was given. It will be obvious from what has been said above that this reversal of the relation between dilator effects in skin and muscle seen with adrenaline and histamine is in harmony with our view that the effect of acetyl-choline is due to arterial dilatation. We have only made one experiment on the point, the volume changes of a skinned limb being compared with those of the opposite whole limb, both having their nervous connexions intact. There was no difference between the response of the two limbs to acetyl-choline such as to indicate a greater effect on skin arteries than on muscle arteries; on the other hand there was no indication that the muscular vessels were preferentially affected, as is so frequently the case with adrenaline and histamine. Hunt's experiments, however, seem to demonstrate that conditions of relative tonus do occur in which the skin vessels show the more pronounced dilatation with acetyl-choline.

Other features of the distribution of the vasodilator effects of these substances are of more doubtful significance. Dale observed erection of the cat's penis after a large hypodermic dose (20 mgm.) of acetyl-choline, and such an effect is certainly not produced by histamine under similar conditions. Hunt recorded the volume of a dog's penis with the plethysmograph and observed expansion with a small dose (0·02 mgm.) of acetyl-choline intravenously. Since the arteries are said to open directly into the cavernous spaces of the erectile tissue, without the intervention of true capillaries, this may be regarded as an unmixed arterio-dilator effect. There is, however, a yet unexcluded possibility that the effect may be central in origin.

## V. Effects of local application of histamine.

We made several attempts to measure changes in the calibre of blood vessels—arterioles and capillaries—in the mesentery by direct observation with the microscope, histamine solution being directly applied to the area under observation. The results were not sufficiently convincing to ourselves to make it desirable to record them. Later experience of the ease with which this vasodilator action is impaired, by temporary interference with the circulation through an organ or exposure to cold, made it clear that satisfactory observations of this kind would need a specially elaborated technique. Results of some significance, however, can be obtained by simple observation with the naked eye of the effects of local application. If a solution of histamine (0·01–0·1 p.c.) in warm saline is brushed across a loop of the intestine of a cat under ether, the local effect on the circulation is complicated by the contraction of the muscular coats of the intestine which the histamine excites. This can be prevented by first painting the whole loop with 0·01 p.c. adrenaline, which inhibits the contractions of the intestinal muscle. The loop acquires a somewhat bluish pallor under the action of the adrenaline. If a drop of 0·1 p.c. histamine solution is then streaked across the loop with a soft brush, the bowel at the line of application becomes gradually red in comparison with that on either side. The reddening had the appearance of a general capillary flush, but the difficulty under the conditions of distinguishing the smallest arterioles from venules made it impossible to say whether the smallest visible arteries were involved in the dilatation. The smallest vessels clearly recognisable as arteries were certainly not dilated.

A clearer result was obtained with the cat's pancreas, which has few or no vessels visible to the naked eye on its surface. The surface of the organ has a very uniform pinkish tint, varying in depth in different animals according as the cells of the acini are loaded with secretory granules or discharged. A half loaded gland shows the effects well. If a pledget of cotton-wool soaked in 0·1 p.c. histamine solution is laid gently on the exposed pancreas, left for 10–20 seconds and then removed, the surface of the gland at the point of application shows a conspicuous diffuse red flush in comparison with the uniform pink of the rest of the pancreas. It seems to us unlikely that such an effect is due to histamine soaking into the gland and locally dilating arterioles. Further evidence of capillary involvement is obtained by allowing the pledget soaked with histamine solution to remain on the gland for 5 or 10 minutes. On now removing it, it is found that the gland underneath it, and in a zone extending a varying distance on either side of it, has become to a pronounced degree œdematous, so that the lobules appear clearly separated and as if embedded in a colourless transparent jelly. At a little distance from the point of application the gland is unaffected. Clearly there has been a local excess of transudation of lymph; the second stage of the effect of histamine has been the production of an abnormal permeability of the capillary walls. This seems to us to give support to the suggestion that the primary local hyperæmia is also due to an action on the capillaries. In the rabbit, in which histamine has no vasodilator action when given intravenously, we observed no effect from local application to the pancreas. The local effect of histamine on the cat's pancreas is clearly analogous to its effect on the lightly scarified human skin, as described by Eppinger(22) and more recently by Sollmann and Pilcher(23). The latter observers found that in solutions as weak as 1 in 100,000 histamine, applied to a scarified spot, caused local erythema terminating in the production of an urticarial wheal. They point out that other vasodilators, such as nitrites, have no such effect, and after a discussion of possibilities, conclude, we think rightly, that the urticaria is due to a specific effect on the permeability of the capillary walls.

## VI. PERFUSION OF SURVIVING ORGANS.

The results of the experiments described in the foregoing pages, though they were often complicated and difficult of interpretation, had appeared to point consistently in one direction. Each of the anomalies in the vasodilator actions of histamine and adrenaline, each of the points in which their actions differed from the more conventional vasodilator action of acetyl-choline, had seemed in turn to find an explanation, if we could suppose that the action of both presented a complex of inhibition of capillary tone and stimulation of arterial tone. Another anomaly of the vasodilator action of these substances remained to be considered—the fact that it had hitherto proved impossible to demonstrate it under conditions of artificial perfusion, although, as we have here shown, it is a purely peripheral effect, independent of the integrity of any nervous mechanism. It is obvious that this also finds its explanation in the above conception if we can suppose that the reactivity of the capillaries is less easily preserved under the conditions of artificial perfusion than that of arterial plain muscle. This is in itself a reasonable supposition, but we were anxious to put our conception to a more direct test. By varying the method of artificial perfusion we have found a combination of conditions which enables us with certainty to obtain the vasodilator effect of histamine in the vessels of the surviving organ, and, further, to make an anatomical separation between the vessels which, under those conditions, it dilates and those which, under identical conditions, it constricts.

The ordinary procedure for perfusing an isolated organ has more than one feature which, in the light of our observations on the conditions favouring the vasodilator effect of histamine *in vivo*, might be held responsible for previous failures to demonstrate it in perfusion experiments. In the first place we found that after a temporary interruption of the circulation the effect disappeared, and was only slowly recovered when the normal circulation was restored. In the ordinary perfusion experiment the vessels of the organ are ligatured, cannulæ are inserted, and the blood is washed out with saline before the artificial circulation is begun. In the second place the customary use of whipped blood is open to criticism for our purpose, since, as Brodie(24) showed, blood so treated has itself a complex activity, including effects which appear to belong to the same general type as that which was later ascribed to histamine, so that an organ perfused with whipped blood might be already in a condition of maximum response to this type of vasodilator effect, and capable only of exhibiting the associated constrictor response with a further dose of this class of poison. A third possibility, that the vessels might need the tonic effect of nervous impulses to enable them to show this type of response, had been excluded by our experiments on the effect of cutting the nerves to a limb in the living animal; but in the living animal, in the absence of nervous control, there is clearly some influence which maintains a vascular tone, as shown by the tonic condition of the vessels in a limb denervated by degeneration. The presence of adrenaline in the circulating blood seems to provide the immediately obvious explanation; but the difficulty at once arises that small doses of adrenaline have a vasodilator action, which is especially pronounced in the vessels of a denervated organ. The conception of a tone maintained by adrenaline and relaxed by a small extra dose of the same substance is certainly difficult. We may reserve its fuller discussion till a later stage. There is one point, however, with which we must deal at once, since it is involved in the procedure we are about to describe. It will be seen that we use the addition of adrenaline to the perfusion fluid as a means of raising the vascular tone and giving scope for vasodilator action. The objection may possibly be raised, that in earlier sections we have given reason for believing, from the analogy of its action with that of histamine, that adrenaline produces its vasodilator effect by action on capillary walls; and it may be suggested that by adding it to the perfusion fluid we should, if this idea is accepted, be producing a state of capillary relaxation which, again on our view, should be unfavourable to the vasodilator action of histamine. It is necessary, therefore, again to emphasize the fact that this conception, so far as it applied to the effect of adrenaline, was concerned with the sudden effect of very small doses. There is good reason to believe that the constrictor effect of any but the smallest doses of adrenaline involves the capillaries as well as the arterioles. The maximum purely depressor dose of adrenaline for the average cat is about 0·005 mgm. Making the rather small allowance of 100 c.c. of blood for such a cat, and assuming that the whole dose remains in the blood, and reaches the site of its action intact, this gives a maximum rise of adrenaline content in the blood of 1 part in 20 millions as the result of such an injection. The concentrations used in our perfusions have been from 1 in 10 millions to 1 in 1 million, and this has been not momentarily attained, but steadily supplied, subject only to the slow diminution occurring during the passage of the whole perfusion mixture through the isolated organ. To obtain the effect which we required we have, therefore, found it necessary to use adrenaline in a maintained concentration much above the maximum at which, when momentarily attained, its vasodilator action appears *in vivo*. Cotton, Slade and Lewis(24) on the human arm with circulation arrested by compression of the artery, obtained effects by local injection of dilute adrenaline (1 in 30,000) which they could only explain by a direct constrictor action on capillary walls, and Langley(28) previously from ocular inspection on local application suggested an action of adrenaline on certain capillaries.

Another point for investigation, suggested by our experiments *in vivo*, was the effect of oxygen supply. The possible bad effects of whipped blood might be avoided by using Ringer's solution as perfusion fluid, giving it the requisite viscosity and content of osmotically active colloid by addition of 6 p.c. gum arabic, as recommended by Bayliss. In doing so we should introduce another factor, that of imperfect oxygen-carrying power, owing to elimination of red corpuscles. This, however, might afford us a further opportunity of discriminating effects on arterial muscle from those on capillary endothelium, which, from the intimacy of its relation to the tissues needing a free supply of oxygen for their metabolism, would probably show earlier impairment of activity from deficiency of oxygen supply. Lastly, by suitable methods of arranging cannulæ, we could eliminate the usual interval between cessation of natural and commencement of artificial circulation. After the first few experiments we have always ensured this practical continuity of circulation, but it has made no perceptible difference in the results. The method of ensuring such continuity has been used in principle before (cf. Bainbridge and Evans(27), Richards and Plant(28)). The perfusions have been carried out on legs and on portions of small intestine. For perfusion of a leg the cannulæ have been inserted into the central ends of the external iliac artery and vein of the other side, the vessels being clamped between the cannulæ and the aorta and vena cava respectively. The aorta was tied beyond the origin of the external iliac artery, all branches of the external iliac artery and vein were tied, and also the profunda femoris vessels. Branches of the aorta and vena cava were tied up to a point above the origin of the inferior mesenteric artery, and the lumbar and abdominal muscles mass-ligatured, as described on p. 137. The cannulæ being connected with the perfusion apparatus, ligatures were drawn tight round the aorta and vena cava near the origin of the iliac vessels, and the clamps on the iliac artery and vein, into which the cannulæ had been inserted, were simultaneously removed. The artificial circulation through the femoral vessels of the opposite side thus began simultaneously with the cessation of the natural circulation. In one case, in which hirudinised blood was used, the cat furnishing the leg for perfusion was also hirudinised and the artificial flow was begun before the aorta and cava were tied, so that not even a momentary interruption of the blood-flow occurred.

Similar arrangements were made for the loop of intestine. The arterial cannula was in this case inserted into the headward end of the aorta, just below the origin of the inferior mesenteric artery. All branches of the aorta above the cannula, up to and including the cœliac axis, were tied. All branches of the inferior mesenteric artery except those supplying the chosen loop of bowel, and all branches of the portal vein except those draining it, were systematically ligatured, and the stomach, the remainder of the small intestine, the spleen, the large intestine were removed after suitable ligaturing. The loop of bowel under observation, still with its normal circulation, was arranged in the plethysmograph, and the cannulæ connected to the perfusion scheme. When all was ready the aorta was ligatured just above the inferior mesenteric artery, and the clamp above the perfusion cannula simultaneously removed. The portal vein was then rapidly incised and an outflow cannula tied into it. When hirudinised blood was used the cat was also hirudinised and perfusion begun before the natural circulation was interrupted, as in the similar experiment on the leg. The effect of interruption of the circulation being thus eliminated, the considerations discussed above had suggested three possibilities for the conditions necessary for demonstrating the vasodilator action of histamine on the artificially prepared organ.

1. It might be possible to demonstrate it if an adequate tone of the vessels were produced by adding adrenaline to the perfusion fluid.

2. The necessary condition might be found in an adequate oxygen-carrying power of the perfusion fluid. We could confer this, and eliminate the defects of whipped blood discussed above, by adding washed red corpuscles to the Ringer's solution or using pure hirudinised blood for perfusion.

3. Both the oxygen-carrying power conferred by corpuscles, and the production of adequate tone by suitable addition of adrenaline, might be necessary.

All three possibilities have been tested on both hind limb and bowel segment, and under each set of conditions we have tested the action of histamine and of acetyl-choline. The conditions were obviously inapplicable to the vasodilator action of adrenaline. Yet a fourth possibility has been tested by using a leg in which the blood vessels had acquired an abnormal reactivity and a natural tendency to tone as a result of degeneration of the nerves to the leg, after section sixteen days previously. The effects of acetyl-choline and histamine were tested during perfusion of this leg with hirudinised blood and with gum-Ringer solution, with and without addition of adrenaline in each case.

In these experiments on perfused organs the dose of the substance being tested was injected by a fine hypodermic needle, pushed through the rubber tubing of the perfusion scheme a short distance above the cannula in the artery. To avoid possible small errors due to changes of viscosity in the fluid reaching the artery, or momentary reduction of adrenaline concentration when this substance was present in the perfusion fluid, doses of histamine were always made up in a sample of the fluid actually being used for perfusion. This could not be done in the case of acetyl-choline when blood was being used, owing to the rapid hydrolysis and consequent loss of activity which it undergoes on standing in contact with blood. The volume of the injection, however, was very small (usually 0·1 c.c.), and the striking nature of the effects of this substance made it impossible in any case to attribute them to the trifling changes of viscosity which might momentarily occur as the result of such an injection.

The following were the results obtained. The effect of acetyl-choline was only tried in the cases mentioned.

1. *Perfusion with oxygenated Ringer's solution containing* 6 *p.c. gum arabic.*

(*a*) Normal leg—Histamine—(in doses from 0·002 mgm. up to 0·1 mgm.) caused contraction of the limb in the plethysmograph and retardation of outflow from the vein.

(*b*) Loop of bowel—Histamine caused the same effect as in the limb—contraction of volume and retardation of venous outflow.

2. *Perfusion with oxygenated Ringer's solution containing* 6 *p.c. gum* + 1 *in* 10 *million to* 1 *in* 1 *million adrenaline.*

(*a*) Normal leg—Histamine—contraction and retardation of outflow. The doses used were 0·01 to 0·03 mgm. Smaller doses had no effect. In the one experiment where very dilute adrenaline (1 in 10 millions) was used, which under these conditions made no difference in the rate of perfusion, 0·02 mgm. of histamine produced a greater reduction of leg volume and retardation of outflow than it had caused earlier in the experiment, where plain gum-Ringer with no adrenaline had been perfused.

Fig. 16 illustrates another experiment. In this case 1 part of adrenaline in 2½ millions was added and the perfusion pressure had to be raised to about 155 mm. of mercury to secure an adequate perfusion rate, showing that a high tone of the vessels had been produced. Nevertheless 0·03 mgm. of histamine still causes contraction of the leg and retardation of the outflow. A slight rise of the perfusion pressure, indicating increased resistance to the flow through the leg, is perceptible as the result of injecting histamine. (It should be remembered in considering this and the following figures that the perfusion is carried out under a static head of pressure with rhythmic interruptions. The changes of pressure recorded are, therefore, small in proportion to the changes of resistance in the organ.)

In only one instance was there any deviation from this type of result. In this experiment, in a preliminary perfusion of the limb with gum-Ringer without adrenaline, 0·002 mgm. of histamine had caused a pronounced constriction of the limb, with a relatively very small change in the rate of outflow, while 0·01 mgm. caused contraction of volume and slower outflow as usual. Adrenaline was then added in the proportion of 1 to 5 millions, and the perfusion pressure raised to keep the flow practically constant. 0·01 mgm. of histamine now had a puzzling effect. The volume of the limb diminished as before; the outflow, however, showed no retardation, but a late and quite obvious acceleration, which showed no tendency to pass off. After an interval of some minutes the dose was repeated with a similar effect—contraction of the limb, and later a further persistent acceleration of outflow. Still later 0·1 mgm. of histamine caused the usual contraction of volume and slowing of outflow. This result is quite anomalous and cannot be explained by any type of vasodilator action. The limb showed a great tendency to become œdematous and increased continuously in volume throughout the perfusion. We found that the plethysmograph fitted rather tightly, and it is possible that the small shrinkage of the limb caused by histamine released fluid which had been accumulating in skin veins, and thus obscured the direct effect of histamine on outflow.

(*b*) Loop of bowel. The addition of adrenaline up to 1 part in 1 million, necessitating a greatly increased perfusion pressure to maintain an adequate flow, reduced the constricting and retarding effect of smaller doses of histamine (0·002 mgm.), but the smallest effective doses remained constrictor in effect, as with the leg.

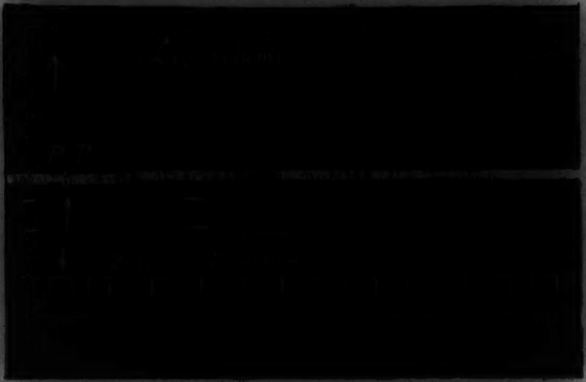


Fig. 16. Perfusion of leg with gum-Ringer and adrenaline (1 in 2½ millions). Effect of 0·03 mgm. histamine.

(*c*) Denervated leg. Histamine caused a similar constriction and retardation of outflow to that which it caused in the normal leg.

Acetyl-choline (0·01 mgm.) caused expansion of the leg and accelerated outflow.

3. *Perfusion with hirudinised blood.*

(*a*) Normal leg—Histamine (0·02 mgm.) caused constriction and retardation, with rise of perfusion pressure.

(*b*) Loop of bowel—Histamine (0·002 mgm.) caused contraction of volume, rise of perfusion pressure and retardation of outflow (Fig. 17 A).

(*c*) Denervated leg—Histamine (0·01 mgm.) caused contraction of volume, rise of perfusion pressure (slight) and retardation of outflow (Fig. 18 A). Acetyl-choline (0·001 mgm.) caused expansion of volume, fall of perfusion pressure (well marked) and accelerated outflow (Fig. 18 B).

4. *Perfusion of hirudinised blood with adrenaline added.*

(a) Normal leg. Adrenaline 1 in 10 millions. Perfusion pressure 180 mm. of mercury. Histamine (0·1 mgm.) caused expansion of the limb, fall of perfusion pressure, and accelerated outflow (Fig. 19).

(b) Loop of bowel. Adrenaline 1 in 1 million. Perfusion pressure had to be raised from about 45 mm. (Fig. 17 A) to about 165 mm. (Fig. 17 B) to secure adequate perfusion rate after addition of adrenaline.

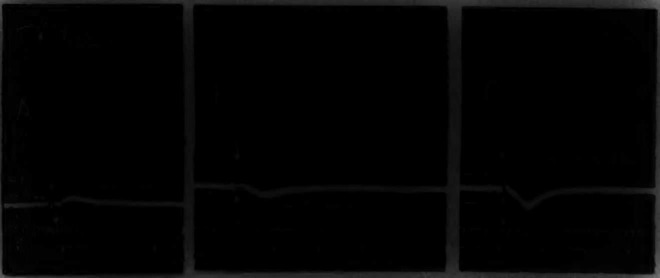


Fig. 17. Perfusion of intestinal loop with hirudinised blood. Oncometer, perfusion pressure, and venous outflow. *A* before, *B* and *C* after addition of 1 part of adrenaline to 1 million of perfusing blood.

Fig. 18. Completely denervated leg perfused with hirudinised blood (no adrenaline added).

Histamine (0·01 mgm.) caused large expansion of volume, fall of perfusion pressure and acceleration of outflow (Fig. 17 B).

Acetyl-choline (0·001 mgm.) caused expansion of volume, fall of perfusion pressure and acceleration of outflow (Fig. 17 C).

Note that acetyl-choline, though in this dose it causes a smaller volume-increase than 0·01 mgm. of histamine, causes a more pronounced fall of perfusion pressure and a greater acceleration of outflow.

(c) Denervated leg. By a mistake the proportion of adrenaline added (1 in million) was much too great, so that even with the highest pressure obtainable with our hydrostatic perfusion the flow through the organ was very slow. Nevertheless the effects with histamine and acetyl-choline were unmistakeable; both now caused expansion of the limb, fall of perfusion pressure, and accelerated outflow.

5. *Perfusion with Ringer's solution containing 5 p.c. gum + washed red corpuscles of cat's blood + adrenaline 1 in 5 millions.*

Normal limb. Histamine (0·02 mgm.) caused expansion of the limb, fall of perfusion pressure and acceleration of outflow.

Fig. 19. Perfusion of normal leg with hirudinised blood to which 1 part of adrenaline in 10 millions has been added. Effect of 0·1 mgm. histamine.

The results of these experiments, which have been presented in this summarized form, show a striking difference between the behaviour of histamine and acetyl-choline. It will be seen that acetyl-choline regularly caused changes indicative of vasodilatation, in all conditions under which it was tested. All that was necessary for the exhibition of this action was the pre-existence of adequate tone in the vessels to give scope for its display. Thus when gum-Ringer was perfused, with the addition of a small quantity of adrenaline to produce tone of the vessels, acetyl-choline showed a well-marked vasodilator action. When the tone was adequate in the absence of adrenaline, as in the denervated limb perfused with blood, acetyl-choline produced again a pronounced vasodilatation.

With histamine the case is far otherwise. It will be seen that we tried all combinations with this substance, using oxygenated gum-Ringer and blood, each with and without addition of adrenaline, for perfusion of the hind leg, with its vessels in normal condition or rendered abnormally reactive by degenerative denervation, or a loop of bowel. In no experiment with Ringer's solution and gum did we obtain any effect on the volume of the organ but contraction as the result of injecting histamine, even when adrenaline was added to the perfusion mixture in such a proportion that a high pressure was needed to maintain an adequate rate of perfusion. In only one case, in which there was evidence of technical error, were the effects on the perfusion pressure and outflow other than those of simple vasoconstriction—rise of pressure and retarded outflow. Similarly, in experiments in which plain blood was perfused, we never obtained with histamine any effects except those of simple vasoconstriction—contraction of volume, rise of perfusion pressure and retarded venous outflow. This was the result even with the denervated limb, in which perfusion with hirudinised blood was begun before the natural circulation was stopped. The animal had been hirudinised, and the volume-reaction of the limb to histamine was tested just before the perfusion was started, and was found to be simple dilatation. As soon as possible after the change from natural to artificial circulation was completed, we tested again the reaction to histamine and observed simple constriction (Fig. 18 A). On the other hand, on organs perfused with blood to which adrenaline had been added histamine never produced any other effects than those of uncomplicated vasodilatation—expansion of volume, fall of perfusion pressure, and accelerated venous outflow.

The experiment on a loop of small intestine, illustrated by Fig. 17, shows especially well the change in response to histamine caused by the addition of adrenaline to perfused blood. In the living animal it is the larger doses of histamine which are specially liable to produce vasoconstrictor effects, even when smaller doses cause only vasodilatation. In this case a pure constrictor effect is produced by 0·002 mgm. of histamine when plain blood is being perfused; after adrenaline has been added a dose five times as large (0·01 mgm.) causes pure vasodilatation.

It is clear, then, that the vascular tone which acetyl-choline relaxes is easily evoked, and is maintained under a variety of conditions. Before these experiments were undertaken the dilator effect of acetyl-choline had been demonstrated by Dale, and more recently by Hunt, on the rabbit's ear simply perfused with cold Ringer's solution. The dilator effect of histamine on the cat's blood vessels is, on the contrary, a singularly evanescent and elusive phenomenon, needing a special combination of conditions for its demonstration in the perfused organ. The question arises, what is the constituent of blood which must be present in the perfusion fluid in order that adrenaline may be able to produce the vascular tone relaxed by histamine? This is answered by the experiment quoted above under heading (5.), in which instead of blood we used a gum-saline mixture to which we added thoroughly washed red corpuscles. This mixture was quite as effective as whole blood in enabling adrenaline to produce a tone which histamine would relax; so that the constituent of blood needed for the effect is the red corpuscles. This we take to mean that the oxygen carrying capacity of a saline solution is inadequate for the purpose.

We find it impossible to conceive of two different kinds of tonus in the same contractile structure, the one, which is relaxed by acetyl-choline but intensified by histamine, being developed under the conditions provided by perfusion with oxygenated saline, and the other, which histamine also relaxes, being produced by adrenaline only when abundant oxygen is provided by the presence of red corpuscles. The only interpretation of the facts which seems to us at all adequate is the supposition that the two substances are acting on different contractile elements in the system of blood vessels. One of these, which is relaxed by acetyl-choline, but stimulated to contraction by histamine, in its resistance to the adverse conditions of ordinary perfusion, and in its ready acquisition of tone with adrenaline perfused in saline solutions, has the properties of arterial plain muscle. The other, which histamine relaxes, is so dependent on an adequate oxygen supply that it does not acquire tone with adrenaline unless red corpuscles are present. Again the walls of the capillaries, with their intimate physiological relation to the oxygen-using tissues, and their susceptibility to the products of imperfect metabolism, seemed to us to be clearly indicated.

When records of the vasodilator actions of acetyl-choline and histamine on the same organ are compared in detail, there is a suggestive difference, well shown in Fig. 17. Attention has already been called to the larger effect of histamine on organ-volume in this experiment, associated with a smaller effect on perfusion pressure and venous outflow than those produced by the dose of acetyl-choline which follows. In this experiment a dose of amyl nitrite was subsequently given, and the proportion between the volume effect and those on pressure and outflow resembled that seen with acetyl-choline, and was similarly contrasted with that obtaining in the effects of histamine. This kind of disparity in the effects of histamine and acetyl-choline has been regularly present in experiments in which the vasodilator effects of both were produced. Histamine, therefore, appears to act on some part of the system of blood vessels relaxation of which has a relatively large effect on the volume of the organ, and a relatively small effect on the resistance to the blood flow. This again is in harmony with the view that it acts on the capillaries with their much larger total sectional area and capacity; the arterioles, with their more important role in determining the resistance to blood flow, being concerned in the vasodilator effects of acetyl-choline, as in those of nitrites.

Since so many different indications appeared to point in the one direction, it was highly desirable to devise an experiment in which the action of the two substances could be tested on the different types of blood vessel separately. We have already described some experiments made by the method of direct application, with results which, though they lend support to the view under consideration, were not decisive. The most conclusive test would be furnished by a perfusion of capillaries only, without intervention of arteries. This has not proved practicable, but we have found no difficulty in carrying out the complementary experiment of perfusion through arteries, including almost the smallest branches still recognisable as such, with no capillaries.

The arteries chosen were the superior mesenteric artery with its branches and their fine ramifications as they pass on to the small intestine. By cutting through the mesentery close to the wall of the intestine, and allowing the blood to drip from the ends of the small arteries thus severed, a preparation is obtained in which perfusion can be carried out through arteries only, and in which the outflow measured is from comparatively fine arterial branches. The arterial cannula is inserted into the aorta as for a perfusion of an intestinal loop. The mesentery is divided above and below the area of arterial distribution to be used for the perfusion. When all is ready the aorta is tied above the origin of the superior mesenteric artery, this artery and its branches are then rapidly washed through with oxygenated Ringer's solution, the mesentery is cut through with sharp scissors as close as possible to the wall of the bowel, and perfusion with hirudinised blood is immediately started. (If the preliminary wash-through with Ringer's solution was not carried out, we found that even hirudinised blood did not prevent the outflow from the small cut arteries becoming obstructed by minute clots.) The preparation is then cut free and suspended from the arterial cannula in a cylindrical glass chamber. The upper end of this is closed by a cork through which pass the cannula and a thermometer, and at its lower end it is narrowed funnel-wise into a tube from which the blood, dripping from the cut edge of the hanging bunch of mesentery, issues in a regular series of drops or a small unbroken stream. The chamber is kept at uniform temperature by an electric glow-lamp placed at an adjustable distance from it, and the preparation is thus protected from both evaporation and cooling. The outflow is recorded in the same way as the venous outflow in our perfusions of whole organs. Doses, as before, are injected into the rubber tubing just above the arterial cannula.

The results are shown in Fig. 20. (*a*) and (*b*) show the effects of small doses of histamine and acetyl-choline when plain hirudinised blood was being perfused. The arteries had little tone and with a low perfusion pressure (65 mm.) the outflow was rapid. Histamine (0·02 mgm.) produces a definite retardation, acetyl-choline a slight acceleration of the outflow. The actual rates, as measured, are 13·4 c.c. per minute before, 16·5 c.c. per minute after injection of 0·001 c.c. acetyl-choline. Adrenaline in the proportion of 1 to 1 million was then added to the perfused blood. The perfusion pressure was raised to about 158 mm. to maintain the rate of perfusion. Histamine (0·01 mgm.) now produces a comparatively weak but still perceptible retardation of flow, 8·5 c.c. per minute being reduced to 7·75 c.c. per minute (Fig. 20 (*c*)). A smaller dose (0·002 mgm.) produced no definite effect, while a larger dose (0·1 mgm.) caused a decided constriction, as shown both by rise in perfusion pressure and retardation of outflow, 10·1 c.c. per minute being reduced to 6·8 c.c. per minute (Fig. 20 (*e*)).

Acetyl-choline under these conditions causes a very obvious dilatation of the arteries, as shown both by increased outflow and fall of the perfusion pressure (Fig. 20 (*d*) and (*f*)). Amyl nitrite (0·01 c.c. emulsified in gum-saline) produced a similar effect to that produced by 0·002 mgm. of acetyl-choline.

Under conditions, therefore, which enable it to produce vasodilatation in the loop of small intestine, histamine causes simple constriction of the arteries supplying that loop, down to their finest anatomically separable branches, while acetyl-choline still produces vasodilatation of arteries thus separated.

The dilator effect of histamine must be on vessels which lie beyond the small arteries, and we are thus led by direct evidence to locate this action on the capillaries, as previously by indirect evidence from the conditions necessary for its demonstration. Of these conditions the need of red corpuscles for the maintenance of capillary tone is intelligible on the lines already indicated. The need for adrenaline requires some further consideration. We must make it clear, in the first place, that we have no evidence that adrenaline is the only substance capable of producing in the perfused organ the vascular tone which histamine inhibits. It is the only substance which we have hitherto tried for this purpose. The point of interest is rather that any such substance should be needed. We have described above an experiment in which we ensured the presence of all the known conditions necessary for an intense vasodilator action by

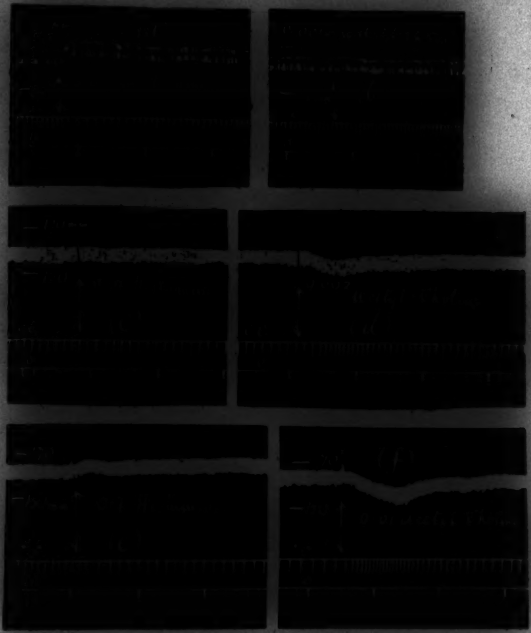


Fig. 20. Perfusion of branches of superior mesenteric artery with hirudinised blood. (*a*) and (*b*) *before*, (*c*), (*d*), (*e*) and (*f*) *after* adding 1 part of adrenaline to 1 million of blood. Perfusion pressure and outflow from cut arterioles.

histamine in the living animal. We used a limb in which the nerves had been cut sixteen days previously, and assured ourselves that, while its natural circulation persisted, it dilated readily with histamine. Lest hirudin in the perfused blood might introduce an unfavourable factor, we injected hirudin into the animal and found that the dilator reaction of the leg persisted. Lastly, lest even a momentary stoppage of circulation should be unfavourable, we began perfusion, from the iliac vessels of the opposite side, before we interrupted the normal circulation. Nevertheless, by the time we were able to make the test, the vasodilator action of histamine in the perfused limb had already vanished, and only a constrictor action remained. Arterial tone was still good, as shown by the vasodilator action of acetyl-choline. The dilator action of histamine did not reappear, the capillary tone was not restored, if our conception is correct, until adrenaline was added to the blood. Why did the change, without even momentary break, from the natural circulation of hirudinised blood to the artificial perfusion of hirudinised blood lead to the loss of capillary tone? Since we are dealing with an organ freed by degeneration from all nervous connexion, the question can be put in another form. What is the substance present in the naturally circulating blood, but absent from blood a short time after it has been shed, which maintains capillary tone in the absence of nervous control? Adrenaline is the immediately obvious suggestion; our observation, that addition of adrenaline to the perfused blood would restore the vasodilator effect of histamine, is at least in accordance with such a suggestion, if it gives it no positive support. When we come, however, to consider how adrenaline, in the concentration in which it exists in the peripheral blood, can play such a part in the living animal, we are met by a difficulty. In the cat, which is the animal on which all our experiments have been made, adrenaline itself, when injected in doses up to about 0·005 mgm., produces a vasodilator action, and we have given reason for believing that this action is of the same type as that of histamine, and therefore presumably an action on capillaries. But experiments in which the output of adrenaline from the suprarenal glands was measured in anæsthetised cats showed that the amount entering the vena cava varied from 0·0008 to 0·0028 mgm. per minute in cats of 2 to 3 kilos body weight (29). Can it be supposed that this slow, steady addition of adrenaline to the circulating blood produces a state of tone, which is temporarily relaxed by suddenly throwing into the blood-stream a further dose of the order of that supplied by the natural secretion in the course of one or two minutes? The conception appears difficult, and is not rendered less so by the fact that large doses of adrenaline (0·05 mgm. and upwards), suddenly injected, cause a simple increase of vascular tone—the vasoconstrictor, pressor effect usually recognised as the characteristic action of adrenaline. There is good reason for believing that capillaries as well as arterioles are involved in the vasoconstrictor action (cf. Langley(26), Cotton, Slade and Lewis(25)). This conception, however, of a tone produced by adrenaline, which an additional dose of the same substance may either slacken or intensify, according to the amount given, is by no means new to anyone who is familiar with the different types of effect produced by adrenaline after ergotoxine has been administered. When, after a dose of ergotoxine insufficient to paralyse completely the vasoconstrictor action of adrenaline, the blood-pressure has been allowed to return to a low level, the first effect of a moderate dose of adrenaline (say 0·05 mgm.) may be to cause a slight further fall, but this is succeeded by a long slow rise of pressure. A second similar dose, given during this rise, produces a more pronounced fall succeeded by a further rise of pressure; and in this way, by successive doses of adrenaline, each producing a more pronounced initial depressor effect, the blood-pressure may be raised to a high level, from which a conspicuous fall follows each injection of adrenaline of the order of 0·05 mgm., while a large injection (say 0·5 mgm.) still causes an immediate further rise. So that it is possible, under the abnormal conditions produced by sufficient ergotoxine to weaken without abolishing the constrictor action, to build up a vascular tone by adrenaline, which further doses may either relax or augment. There is, therefore, no insuperable difficulty in the supposition that, on a lower scale of dosage, the double action of adrenaline may be manifested, in the normal animal, by an analogous paradoxical effect, a further dose relaxing the tone which the same substance in steady low concentration has produced. The possibility needs further experimental exploration before it can be accepted as a satisfactory explanation of the phenomenon with which we are dealing; but it cannot be set aside as inherently incredible, or contrary to all experience.

## VII. Remarks.

This question of the maintenance of vascular and especially of capillary tone, apart from nervous control, is one possessing great possibilities of practical interest. The probability that some products of cellular activity produce a relaxation of capillary tone, such as we have here identified as the action of histamine, has long been recognised, and has frequently been associated with the increased rate of blood-flow through an organ, accompanying acceleration of its metabolism(30). The appearance of substances, having what we call the "histamine" type of action on the vascular system, in blood subjected to mechanical violence or during the process of clotting (Brodie), and the fact that extracts of so many organs and tissues contain substances exhibiting this type of activity, must have some physiological significance. Barcroft and Piper(31) attributed the vasodilatation, occurring in the submaxillary gland after injection of adrenaline, to some product of the increased metabolism of the gland cells. It is perhaps significant that they found this action to be abolished when the gland was subjected to prolonged perfusion with cold saline, adrenaline then producing only its vasoconstrictor effect after the normal circulation had been restored. We have found that similar treatment of a leg abolishes the dilator action of histamine, and have attributed the change to depression of the reactivity of the capillaries. If it be the case that metabolic products with histamine-like action are constantly arising in the tissues, their production accelerated by every excitatory or injurious influence which intensifies metabolic activity, the capillary endothelium must as constantly be exposed to influences tending to relaxation of its tone. In a forthcoming paper, dealing with the action of large doses of histamine, we shall show how the simultaneous production of such action in an intense form throughout the body rapidly produces a condition of irremediable circulatory collapse, due to the stagnation of practically all the blood in the peripheral vessels. The continuous formation of metabolic products with histamine-like action must surely lead to a similar shock-like stagnation of blood in the relaxed capillaries, unless its influence were constantly neutralised and capillary tone upheld by nervous impulses, or by the continuous addition to the blood of some antagonistic principle. It seems to us that a more general investigation of the means by which this balance is normally maintained, and of the conditions leading to its upset in the direction of general capillary relaxation, is likely to give information having an important bearing on traumatic shock and allied conditions (cf. Cannon(32)). There is very little evidence concerning nervous control of capillary tone in mammalia, though there is some evidence of its existence in the frog. The possibility that maintenance and restoration of capillary tone may be among the most important of the functions of adrenaline is an attractive subject for speculation, but as yet no more.

A word may be added as to the relations between the vasodilator effects here under consideration and the different types of innervation of blood vessels. As pointed out by Dale, and more recently by Hunt, the effects of acetyl-choline, like those of muscarine or pilocarpine, have in most directions an obvious likeness to those of the nerves called parasympathetic in Langley's classification of the autonomic system. But its general dilator action on systemic arteries has no parallel in parasympathetic innervation. Hunt tests the possibility of an association between vasodilatation by acetyl-choline and the antidromic action of sensory nerves. He rejects it on the ground that atropine does not abolish the latter effect, while it immediately suppresses that of acetyl-choline. The argument has not much weight, as he admits, since the vasodilator effect of the chorda tympani, which acetyl-choline obviously simulates, is but little affected by moderate doses of atropine. We have frankly abandoned the attempt to establish a connexion between the vasodilator effects of the three substances which we have specially studied and any system of innervation. From analogy with its other effects we should expect the vasodilator action of adrenaline to be represented in the sympathetic innervation of the vessels. The evidence for the existence of vasodilator effects of true sympathetic nerves is, however, scattered and meagre; the dilatation of the coronary arteries, the flushing of the mucous membrane of the dog's mouth, described by Dastre and Morat(33) as caused by stimulating the cervical sympathetic nerve, and certain relatively weak vasodilator effects of sympathetic nerves seen after ergotoxine, seem to be the only definite examples. On the other hand the vasodilator action of small doses of adrenaline so closely resembles in its distribution, and in the conditions favouring its demonstration, the more powerful effect of histamine, that it seems possible that the two substances have, in this direction, a common type of action, and that this is something additional and extraneous to the sympathomimetic action of adrenaline.

The only hint given by our experiments of connexion between innervation and the effects of these substances, is the enhancement of the effects of all three produced by denervation. We take this, however, simply to mean that the reactivity of the normally innervated vessels is restricted by the effect of tonic impulses from the nerve centres; only when these are cut off, and when the stimuli aroused by nerve section and by the earlier processes of degeneration have passed off, can the contractile elements in the blood vessels exhibit their unhampered response to these drugs. This view is supported by the fact that denervation similarly enhances the vasodilator effects of amyl nitrite and nitro-glycerine, which have never been associated with any type of innervation.

For the sake of brevity and emphasis we have throughout this paper spoken of the vasodilator effect of histamine (and by analogy that of adrenaline) as due, in our opinion, to action on capillary walls; of the constrictor effect of histamine and the dilator effect of acetyl-choline as due to action on arterial muscle. It is important, therefore, to make it clear that no sharp distinction is warranted by our evidence. What we have actually on evidence is that the anatomically separable arterial branches are stimulated to constriction by histamine and adrenaline, to dilatation by acetyl-choline; that, therefore, the dilator effect of histamine which, under identical conditions, predominates in the whole organ, must be an effect on vessels more peripheral than these. The conditions necessary to produce this vasodilator effect support the view that it is mainly an effect on capillaries. The possibility is not directly excluded that it may spread on to the smaller arteries, overlapping with the vasoconstrictor action in such a way that there is a zone in which one or other action is the effective resultant according to dosage. At the same time there is no evidence of such overlap in the case of histamine, and such a conception would renew our original difficulty of reconciling a relaxing effect on arterial muscle with the stimulant action of histamine on all kinds of plain muscle, in any dose in which it is effective at all.

The simpler conception which regards the dilator effect of histamine as an action on capillary walls, and its action on the plain muscle of the arteries as being invariably to increase its tonus, accords better with the known facts. In the case of adrenaline the position is different. The evidence for the more peripheral location of its dilator action is indirect as yet, and there is evidence of overlapping of the two types of action, since the tone of the capillaries also is increased by larger doses and apparently by the smallest effective concentrations if maintained. It may well be that the vasodilator action of a sudden small injection of this substance affects both the capillaries and the smallest arteries, while the constrictor effect of a bigger dose spreads from the arteries to involve the capillaries as well. The reversal of its action on the lung vessels with increase of dosage (Tribe(34)) may possibly find its explanation along these lines. The whole question of the relation of these opponent effects of adrenaline needs further investigation; even the relation of the normal dilator action of small doses, to that produced by doses of any dimensions after an adequate amount of ergotoxine has been administered, requires clearer definition. In the case of acetyl-choline our evidence only entitles us to say that it possesses an inhibitor effect on arterial muscle which sufficiently accounts for its vasodilator action along conventional lines; it *may* directly relax capillaries as well, but we have no evidence of its doing so.

We are aware that the view which we have here put forward as to the manner of action of small doses of histamine necessitates some modification of the accepted teaching, which localises the peripheral resistance, by which the arterial pressure is maintained, almost wholly in the smaller arterial branches, and regards the resistance met in the capillaries as almost negligible from this point of view. Local variations of capillary tone have been described (cf. Roy and Graham Brown (19) who cite earlier literature) and have been credited with importance in directing the distribution of the blood under conditions of low blood-pressure (Langley (35)). So far as we are aware, however, the conception of the intrinsic tone of the capillary wall as playing an important part in regulating the general peripheral resistance, and maintaining the arterial blood-pressure, does not enter into any accepted system of physiological teaching. Yet this is implicit in the view which we have put forward; for the fall of arterial blood-pressure caused by a small dose of histamine has all the characteristics of a fall caused by the recognised agents for lowering the peripheral resistance. It is not a "volume" effect, due simply to an increased capacity of the system; if it were so the output of the heart must be lessened during the fall of blood-pressure, owing to diminished venous inflow, whereas it is somewhat increased. The question which arises is whether the accepted teaching, locating the peripheral resistance in the arterioles, and attributing variations in its strength entirely to variations in the tone of arterial muscle, is based on evidence so conclusive as to nullify the evidence which we have put forward. It does not seem to us that it is so. There is evidence that the total sectional area of the capillaries is very much larger than that of the larger arteries, though it may be doubted whether the measurement of the rate of flow through particular capillaries, exposed for microscopic observation, and the assumption that the general rate throughout the body is of the same order, can lead to a quantitatively correct estimate of the relation. There is evidence, again, that the pressure in capillaries accessible for its measurement is usually nearer that of the veins than that of the arteries. We find no evidence, however, to warrant the assumption that the steep fall of pressure in the arterioles suffers an abrupt flattening, becomes in fact almost horizontal, at a sharp line of demarcation between the smallest arterioles and the first capillaries. We find nothing to exclude the view that a general tone of the capillaries, if it exists, will play an important part in determining the peripheral resistance, and that a sudden relaxation of this tone will cause a fall of blood-pressure of the vasodilator type. It is, however, legitimate to suppose that such a relaxation would have more effect on the volume of the organs involved, in proportion to the diminution of resistance effected, than a relaxation of the tone of the corresponding arterioles. Under the uncomplicated conditions of artificial perfusion we have shown that this expectation is realised. Whether this interpretation of the facts here recorded be correct or not, it is clear that the results obtained by artificial perfusion of isolated organs lead to an incomplete and misleading conception of the action of histamine and similarly acting substances, if the experiment is made in the ordinary way. Other factors are concerned in the action of these substances on the circulation than those which have hitherto been appreciated, and we are convinced that the physiological importance of these factors will be increasingly apparent.

## SUMMARY.

Our main conclusions may be summarized as follows:

1. The vasodilator actions of histamine, adrenaline and acetyl-choline are purely peripheral effects on the blood vessels, independent of the integrity of any nervous connexion. The blood vessels are usually rendered abnormally sensitive to these actions by complete degeneration of the nerve-supply.

2. The vasodilator action of acetyl-choline is dependent on the state of tone and reactivity of the arteries.

3. The vasodilator actions of histamine and adrenaline undergo parallel variations with changes in the condition of the blood vessels. These variations are independent of the tone of the arteries, and both actions are favoured by a condition which we regard as indicative of tone and reactivity of the capillaries. The vasodilator actions of the two substances resemble one another further in their limitation to certain animal types and their relative prominence in the vessels of different organs.

4. The vasodilator action of histamine can be regularly demonstrated on artificially prefused organs of the cat if the perfusion fluid contains red blood-corpuscles and a small proportion of adrenaline. If either is omitted histamine produces only vasoconstriction, as in earlier experiments. Even when both are present histamine produces constriction of arteries separated from their peripheral distribution. Acetyl-choline produces dilatation in the perfused organ or separated arteries under the simplest conditions.

5. We conclude that the vasodilator effect of histamine, and probably that of adrenaline, are due to relaxation of the tone of the capillaries, while that of acetyl-choline is due to action on arteries. Histamine has

a constrictor effect on arteries; the better known vasoconstrictor effect of adrenaline probably involves both arteries and capillaries.

6. No exact line can be drawn at present as to the point where the characteristic arterial reaction gives way to the characteristic capillary reaction.

7. It is suggested that the current conception of the peripheral resistance to blood flow, as determined almost exclusively by the tone of the arterioles, allows too little importance to capillary tone as a factor.

8. No connexion has been established between the vasodilator actions of histamine and adrenaline and any system of nerves.

9. The possibility is considered that substances with histamine-like action are produced by activity or injury of the tissues, and the bearing of this on physiological vasodilatation and shock is discussed.

## BIBLIOGRAPHY.

(1) **Dale and Laidlaw.** This Journal, **41**, p. 318, 1910; **43**, p. 182. 1911.
(2) **Guggenheim.** Therap. Monatsch. **26**, p. 795. 1912.
(3) **Mautner and Pick.** Münch. med. Woch. **62**, p. 1141. 1915.
(4) **E. Mellanby.** Quart. Journ. Med. **9**, p. 165. 1916.
(5) **M. R. C. Memorandum.** B. M. J. 1917, i, p. 381; Lancet, 1917, i, p. 502.
(6) **Hartman and Fraser.** Amer. Journ. Physiol. **44**, p. 353. 1917.
(7) **Dale.** Journ. Pharm. and exp. Ther. **6**, p. 147. 1914.
(8) **Hunt.** Amer. Journ. Physiol. **45**, pp. 197, 231. 1918.
(9) **Richards and Drinker.** Journ. Pharm. and exp. Ther. **7**, p. 467. 1915.
(10) **Bayliss.** Proc. Roy. Soc. B. **89**, p. 380. 1916.
(11) **Gruber.** Amer. Journ. Physiol. **45**, p. 302. 1918.
(12) **Gruber.** Ibid. **43**, p. 530. 1917.
(13) **Vincent and Sheen.** This Journal, **29**, p. 242. 1903.
(14) **L. Hill.** Schäfer's Text-Book of Physiol. **2**, pp. 136, 137.
(15) **Cannon and Lyman.** Amer. Journ. Physiol. **31**, p. 384. 1913.
(16) **Bayliss.** This Journal, **26**, p. 173. 1900.
(17) **Goltz.** Pflüger's Arch. **9**, p. 174. 1874.
(18) **Luchsinger.** Ibid. **14**, p. 391. 1877. **Puelma and Luchsinger.** Ibid. **18**, p. 489. 1878.
(19) **Roy and Graham Brown.** This Journal, **2**, p. 323. 1879.
(20) **Barger and Dale.** Biochem. Journ. **2**, p. 240. 1907.
(21) **Hoskins, Gunning and Berry.** Amer. Journ. Physiol. **41**, p. 513. 1916.
(22) **Eppinger.** Wien. med. Woch. **23**, p. 1414. 1913.
(23) **Sollmann and Pilcher.** Journ. Pharm. and exp. Ther. **9**, p. 309. 1917.
(24) **Brodie.** This Journal, **26**, p. 48. 1900.
(25) **Cotton, Slade and Lewis.** Heart, **6**, p. 223. 1916.
(26) **Langley.** This Journal, **27**, p. 248. 1901.
(27) **Bainbridge and Evans.** Ibid. **48**, p. 278. 1914.
(28) **Richards and Plant.** Journ. Pharm. and exp. Ther. **7**, p. 485. 1915.
(29) **Stewart and Rogoff.** Ibid. **8**, p. 479. 1916.
(30) **Barcroft.** The Respiratory Function of the Blood, Cambridge. 1914.
(31) **Barcroft and Piper.** This Journal, **44**, p. 359. 1912.
(32) **Cannon.** Journ. Amer. Med. Assoc. **70**, p. 611. 1918.
(33) **Dastre and Morat.** C. R. Acad. d. Sci. **91**, p. 393. 1880.
(34) **Tribe.** This Journal, **48**, p. 154. 1914.
(35) **Langley.** Ibid. **41**, p. 483. 1911.

# THE DISTRIBUTION OF BLOOD, GLYCOGEN AND FAT IN THE LOBES OF THE LIVER[1]. By V. B. DOWLER, B.A. (Toronto), M.Sc. (McGill) and V. H. MOTTRAM, M.A. (Cantab.).

(*From the Physiology Department, McGill University, Montreal, Canada.*)

That the secretory portions of the different lobes of the liver approximate closely in their chemical composition is a natural assumption. The organ receives so large a blood supply from so many sources that it is difficult to conceive that any one lobule should differ much from any other. Individual and closely adjacent cells undoubtedly differ, as may easily be seen in preparations made to demonstrate either glycogen, fat, iron, or mitochondria. But the unprejudiced observer would probably say that these differences average out over a large area. Certainly that has been the impression of one of us as the result of examining numbers of liver sections in the past twelve years.

None the less there are observations which suggest that this natural assumption is false. For example: often on perfusing a liver with a colourless solution portions will be seen that remain obstinately red, and it will require gentle massage to clear them. Such patches have no apparent gross anatomical relation to the rest of the liver, and might be explained as due to a local clot or perhaps a localised vaso-constriction. Should such a state of affairs be common in the intact animal, it is easy to see how the composition of one lobe at any time might differ from that of another. Gilbert and Villaret(1), however, using a gelatin injection method, claim that the injection mass penetrates equally all parts of the liver even when, instead of the portal vein, they use one or other of the mesenteric veins for injection purposes. Bauer(2), using the same method, agrees. Consequently, if it had not been for the work of Sérégé(3, 4, 5, 6), and the more recent and elaborate work of Bartlett,

---

[1] Based on a thesis presented by V. B. Dowler for a M.Sc. degree, McGill University, 1916.

**Corper** and **Long**(7), the uniformity of the lobes of the liver would not have been questioned.

**Sérégé**(8) by three independent methods (urea and glycogen estimation at different periods of digestion and the circulation time of potassium ferrocyanide) concludes that the circulation through the right lobe of the liver is more rapid than that through the left, with the result that the latter parts with its stores of urea and glycogen more slowly, and that, further, in the early hours of digestion the digestion products predominate in the left lobe, later (at the sixth hour) in the right lobe, and finally, at the eighth hour are equally distributed. **Bartlett, Corper** and **Long**(7), using a different technique, come to a conclusion that confirms and extends **Sérégé's** view. They inject the various branches of the portal vein with emulsions of olive oil in defibrinated blood, and also study the absorption and appearance in the liver of copper salts from fistulæ of various parts of the intestine. They state that blood from the stomach, spleen, upper jejunum and rectum flows principally to the left and central lobes, while that from the lower jejunum, ileum and most of the colon, flows to the right lobe. Consequently the relative amounts of glycogen and amino-acids in the different lobes of the liver must vary from time to time according to the stage digestion has reached. At one time the left lobe may be favoured, at another the central or right lobe. By chance it may happen that the compositions of all three lobes are identical. But the probabilities are great that there is a difference, and that it is not justifiable to use the analysis of one lobe of the liver as control for that of another. We have thought it worth while to make further experiments to see if there be any escape from this deduction. We have injected the hepatic artery with an emulsion of oil in defibrinated blood to test the distribution of arterial blood, and caused slow physiological infiltrations of the liver with fat and rapid ones with glucose. In every case the non-equivalence of the various lobes has been substantiated. Further, the distribution of the various substances cannot be predicted; the partition takes place in an apparently haphazard way.

*Distribution of arterial blood.* The general plan of the experiment was to anæsthetise the animal (cat or dog), open the abdomen and insert a cannula into the hepatic artery. An emulsion of olive oil in defibrinated blood procured from the animal itself, was injected through this cannula, and the animal was immediately bled to death. The liver was then excised and portions of it at once fixed in a fluid consisting of 5 parts of glacial acetic, 10 parts of 40 p.c. formaldehyde solution and 85 parts of Ringer's solution. Histological preparations were made, stained for fat with Sudan III and the fat globules in a given area counted.

The criticisms of such a procedure are many. The abdomen was opened, thus altering the pressures in the different lobes of the liver. The hepatic artery was ligated, thus cutting off for some time before the injection of the oil emulsion, the arterial blood supply of the liver and partially asphyxiating the liver cells. Doubtless, too, the hepatic plexus was stimulated during the operation with an ensuing possibility of unequal vaso-constriction in the liver lobes. Injection from a syringe is but a crude imitation of an arterial flow of blood. The amount of fat emboli near the hilus of the gland is sure to be different from that of the periphery and, finally, intracellular fat may easily be mistaken for fat in the capillaries.

Some of these criticisms we could obviate. To allow any accidental vaso-constriction to pass off, a wait of twenty minutes between the insertion of the cannula into the hepatic artery and the injection of the emulsion was made. Material for histological preparations was taken from hilar, peripheral and central parts of the lobes and the results averaged. 10 portions of each liver were used and 7 slides made from different parts of each portion in the case of cats and 5 in the case of dogs, making 70 and 50 specimens respectively. Counts were made of the oil emboli by means of a mechanical stage and for comparison calculated for an area of 10 sq. mm. By such a procedure it is assumed that local and accidental variations were averaged out. To aid in the distinction between intracellular and intracapillary fat the sections were counterstained with Delafield's hæmatoxylin, and in any case of difficulty (as is almost invariably so with cats) high power instead of low power counts were made. Finally any experiments about which we had the slightest doubt of the operative technique were rejected: over 40 p.c. of the experiments made were discarded on such grounds.

In view of criticisms such as the above, we should lay but little stress on the results did they not confirm those of more easily controlled experiments. Table I gives the average results for the number of fat globules per 10 sq. mm. of histological preparation in the right, the central and the left lobes of the livers investigated. The first five experiments detailed were made upon cats; 28 counts were made of the fat globules in the left lobe, and 21 in each case in the right and central lobes. With the dogs the figures are 20, 15 and 15 for the left, the right and central lobes respectively. In an actual experiment (No. 1, cat) the figures run: right lobe, main mass 61·3, spigelian 19·3, caudate 49·1;

central lobe, periphery 53·3, hilus 49·1, central 53·1; and left lobe, periphery 154·1 and 54·7, hilus 42·3 and 45·2. Averaging we get: right lobe 43·2; central 51·8 and left 74·1.

TABLE I.

| | | Right lobe | Central lobe | Left lobe | Lobe with most emboli |
|---|---|---|---|---|---|
| Cats | 1 | 43 | 52 | 74 | left |
| | 2 | 32 | 33 | 35 | equal |
| | 3 | 24 | 19 | 17 | right |
| | 4 | 27 | 26 | 43 | left |
| | 5 | 42 | 42 | 23 | right and centre |
| Dogs | 1 | 38 | 35 | 45 | left |
| | 2 | 19 | 17 | 20 | equal |
| | 3 | 26 | 32 | 41 | left |
| | 4 | 23 | 29 | 75 | left |
| | 5 | 73 | 34 | 42 | right |

As will be seen from the figures there is a marked variation within the individual lobes, nor do the hilar portions obtain more emboli than the peripheral portions. And from the remaining experiments given in Table I it is seen that we cannot predict which lobe will contain the greatest amount of injected fat. In two experiments the amount is roughly the same in each lobe, in two the right lobe shows most, in five the left, and in one the right shares that position with the central lobe. There is thus, in these experiments, no evidence for the equivalence of the different lobes, or of different parts of the same lobe. If then injection oil emulsions are distributed unevenly between the different lobes and parts of the lobes there is a probability that arterial blood is distributed according to no regular and proportional plan.

*Distribution of glycogen after a meal of cane-sugar.* Any difference in the nature or supply of the blood to the different lobes of the liver as postulated by Bartlett, Corper and Long(7) and suggested by the above experiments should result in an inequality of the distribution of glycogen after a carbohydrate meal. The blood from the various parts of the alimentary tract does not thoroughly mix in the portal vein, so that the blood which passes to one lobe may easily be richer in a certain product of digestion than that to another. A heavy meal of cane-sugar after a preliminary fast may be expected to bring out such a difference in distribution of glycogen in the liver for the main avenue of infiltration of the liver will be by the portal vein. In view of the fact that the blood sugar rises after a carbohydrate meal, not only in the portal system but in the general system, a difference in the partition of arterial blood in the liver, which we have shown is probable, will either accentuate or decrease the inequality produced by the unequal distribution of portal blood, but can hardly mask it. When the alimentary tract has been at rest for some time we might expect an equality in the distribution of a digestion product in the lobes of the liver, but probably at no other time.

The point has been investigated by Sérégé (3, 4 and 5) and by J. J. R. Macleod (6). As stated above Sérégé claimed that the digestion products varied in the right and left lobe at different periods of digestion. Macleod, however, using a technique which was a great improvement on that of Sérégé, found that there was no large variation in glycogen content between the lobes of the liver after a cane-sugar meal. The variation lay between 5 and 10 p.c. and was due to unavoidable errors in the method used in the estimation of glycogen (Pflüger's).

We have thought it worth while to repeat Macleod's experiments. Dogs after a preliminary fast were fed with a strong solution of cane-sugar through a stomach tube. 3 to 5 hours later (in one case 16 hours later) the animals were anæsthetised as gently as possible. The liver was excised and perfused with ice-cold 0·9 p.c. sodium chloride solution. Portions from the different lobes were taken, weighed and their content of glycogen estimated by Pflüger's method. The whole procedure from the time of giving the anæsthetic till the liver was on the water-bath occupied only half-an-hour, a time in which the diastatic ferments could have but little influence on the glycogen. Slight, but immaterial variations of the technique were made, principally with the object of rapidly removing the pigment from the precipitated glycogen. For the estimation of the resultant glucose Allihn's gravimetric tubes were used.

TABLE II. Content of the liver lobes of dogs in glycogen per 10 grams weight.

| | Hours after cane-sugar meal | Right | Centre | Left | Greatest difference | Percentage of largest amount | Lobe with largest amount |
|---|---|---|---|---|---|---|---|
| 1 | Fasting | 0·540 g. | 0·540 g. | 0·523 g. | 0·017 g. | 3·1 | right and centre |
| 2 | 3¼ | 0·794 | 0·860 | 1·168 | 0·374 | 32·0 | left |
| 3 | 3¼ | 0·560 | 0·412 | 0·325 | 0·235 | 41·9 | right |
| 4 | 3¾ | 2·000 | 1·839 | 1·737 | 0·263 | 13·2 | right |
| 5 | 4 | 0·243 | 0·515 | 0·672 | 0·429 | 63·8 | left |
| 6 | 5 | 1·405 | 1·103 | 1·251 | 0·302 | 21·4 | right |
| 7 | 16 | 1·130 | 1·077 | 1·010 | 0·12 | 10·6 | right |

In Table II the amounts of glycogen present in the right, the centre and the left lobes are given, calculated to 10 grams of liver substance. To bring out the differences in amounts between the lobes, the greatest difference between two lobes of the same liver is expressed in percentage of the largest amount. It will be seen that in a fasting dog the amounts agree to within 3 p.c. and in the dog killed 16 hours after the meal of cane-sugar to within 10 p.c. In the animals killed from 3 to 5 hours after the meal the disagreement runs from 13 p.c. to 63·8 p.c., a disagreement that surely cannot be laid to the charge of faulty technique; in no case does the central lobe obtain the largest amount of glycogen; in two cases the left lobe is favoured, and in three cases the right lobe. There is no apparent relation between the time that elapsed after the meal and the lobe that has the largest amount of glycogen. As there was probably no delay of the sugar solution in the stomach, the latter being empty, the animals were at the height of digestion and absorption. The infiltration of the liver with glycogen was rapid, and, as far as the experiments go, the distribution of the glycogen was irregular and according to no fixed plan. In such experiments it is not reasonable to accept the analysis of one lobe of the liver as a control for that of another.

*Distribution of fatty acid in an infiltrated liver.* So far we have been dealing with changes in the content of the liver that took place rapidly. We have overburdened the lines of communication with a possibility of local congestion and stasis. It was natural to assume that a slow change in liver content would result in an equality of distribution of the various proximal constituents. For this purpose we used rabbits and caused an infiltration of the liver with fat by means of a fast from one to three days' duration. As has been shown by one of us (9), the liver has its store of fat considerably increased (not only relatively but actually) in the early stages of a fast. During the second 24 hours this added fat largely disappears, and a second pulse of infiltration follows on this disappearance during the third period of 24 hours or later. As this mobilised fat approaches the liver either by way of the portal vein or the hepatic artery, and as the mobilisation lasts a considerable time, there is every opportunity for the fat to be equally divided between the lobes of the liver.

The animals, after the preliminary fast, were killed, the liver excised and perfused for five minutes with ice-cold 0·9 p.c. sodium chloride solution. Portions of from 10 to 15 grams weight were taken from the three lobes, any visible fat (there very rarely is any) and the large blood vessels dissected away, and the remainder minced, weighed and transferred to pressure bottles. The subsequent analysis closely followed the method of fatty acid estimation devised by one of us (10). Using this method the disagreement between parallel estimates on the same liver tissue is about 1 p.c. Consequently the somewhat grave uncertainty with regard to glycogen estimations (in which the error may be in the neighbourhood of 10 p.c.) is not met with in these experiments.

TABLE III. Content of the rabbits' liver-lobes in fatty acid per 10 grams weight after a fast.

| | Days' fast | Right | Centre | Left | Greatest difference | Percentage difference | Lobe with largest amount |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 0·3771 g. | ·2338 g. | ·3355 g. | ·1433 g. | 38·2 | right |
| 2 | 0 | 0·2731 | ·3061 | ·3163 | ·0432 | 13·7 | left |
| 3 | 1 | — | ·4589 | ·4050 | ·0539 | 11·7 | central |
| 4 | 2 | 0·4137 | ·3898 | ·3605 | ·0532 | 12·9 | right |
| 5 | 2 | 0·3755 | ·3024 | ·2721 | ·1034 | 27·5 | right |
| 6 | 3 | 0·5009 | ·6459 | ·5583 | ·1450 | 22·4 | central |
| 7 | 3 | 1·2779 | ·9650 | ·8706 | ·4073 | 32·9 | right |
| 8 | 3 | 0·6133 | ·5408 | ·5016 | ·1117 | 18·2 | right |
| 9 | 3 | 0·3505 | ·3672 | ·3479 | ·0193 | 5·2 | central |
| 10 | 3 | 0·4088 | ·4271 | ·5086 | ·0998 | 19·6 | left |

The results are set out in Table III. The fatty acid content of the different lobes of the liver is calculated to 10 grams of liver substance and the differences to be met with in any one liver calculated in percentage of the largest amount found. As will be seen at a glance the story is the same as before. The fatty acid is unequally divided between the three lobes, and in the ten experiments the variation runs from 5 p.c. to 38 p.c. The disagreement in the mean is 20 p.c. Surely such large variations cannot be due to a failure in the technique of a method which in ordinary hands is accurate to 1 p.c. The conclusion to which we are forced is that the percentage composition of the three lobes of the same liver varies widely. Nor can we predict which lobe of the liver will have the greatest amount of fat. In five experiments the right lobe has the preponderating amount; in three, the central lobe; and in two, the left lobe. Of the livers showing an undoubted infiltration three have the maximum in the central lobe, two in the right and one in the left lobe.

## SUMMARY AND CONCLUSION.

Evidence has been produced that the lobes of the liver, whether of dog, cat or rabbit, have different and unpredictable compositions. The result of injection of oil emulsions into the hepatic artery suggests that the partition of arterial blood in the liver is irregular, as was shown to be the case with the portal blood by Bartlett, Corper and Long. The distribution of glycogen in the lobes of the liver after a carbohydrate meal is unequal and irregular, confirming, in part, the results of Sérégé with glycogen and urea, and of Bartlett, Corper and Long with copper absorbed from intestinal fistulæ; but conflicting with their results in being unpredictable as regards the magnitude of the amount distributed to a definite lobe. The distribution of fatty acid among the lobes of the liver during a relatively slow infiltration with fat also is according to no definite plan. The liver tissue does not receive fat in proportion to its mass, nor is one lobe always favoured at the expense of the others. In one experiment the right lobe, in another the central lobe, or in a third the left lobe will contain most fatty acid. The variation between the lobes exceeds the error of experiment in the mean by twenty times.

There is strong evidence, then, from rapid injection under artificial conditions, and from rapid and slow infiltrations under physiological conditions, that material brought to the liver by the blood stream is distributed according to no regular plan. Consequently we can never assume that all parts of the liver are working at the same rate or upon the same material, nor are we justified in assuming that one lobe or portion of a lobe can be used as a control for another lobe or portion of a lobe. The nearest approach to an exception to this generalisation is the liver of a fasting dog.

The probable factors that influence the partition of metabolic products between the different parts of the liver are localised constriction (whether passive, due to postural pressure, or reflex), incomplete mixing of the blood in the portal vein and varying potentiality in the cells for a certain piece of work. Thus it is quite possible that the liver cells with their multiform activities do not exercise them all at the same time. The idea has often been mooted that the glycogenic function of the liver antagonises its function in fat metabolism (11), and although this idea as regards the whole of the liver is, according to one of us, based on a misconception of the meaning of fatty infiltration of the liver, it is none the less possible that it is true of individual cells. It is difficult to understand how a cell so grossly infiltrated with fat that its nucleus is pressed out of shape and its protoplasm reduced to a mere annulus can exercise its glycogenic, deaminising, detoxicating and antibody and bile producing functions. Yet a liver with a very high grade of infiltration seems to function normally, possibly because all its cells have not reached the same state of infiltration. Only in extreme grades of infiltration does the function of desaturation of fats appear to lag behind what might have been expected (12). Of such a possible local activity and rest we have examples in other glandular organs. In a verbal communication to one of us the late Professor Brodie said that in the kidney, even at the height of secretion, some glomeruli are apparently not at work. In an active salivary gland, too, it is not unusual to find alveoli of resting cells.

To such a division of labour among large masses of cells our experiments on fat infiltration testify. The invasion of the liver is slow, consequently local constrictions and dilatations, passive or reflex, will have been relieved and changed in locus. The invasion takes place along two different channels, the portal vein and the hepatic artery. The blood, bearing the fat, must have been adequately mixed in its multitudinous circulations through the system. The first two factors discussed above as causing unequal partition of metabolic products among the lobes of the liver are thus probably eliminated. There remains the third: the varying potentiality of the individual cells for a given piece of work at a given time. For the present, however, we put this forward as a hypothesis to explain our anomalous results.

## REFERENCES.

(1) **Gilbert and Villaret.** Arch. d. Méd. exp. 21, p. 273. 1909.
(2) **Brissaud and Bauer.** Journ. de l'Anat. 45, p. 1. 1909.
(3) **Sérégé.** C. R. d. l. Soc. d. Biol. 54, p. 200. 1902.
(4) **Sérégé.** Ibid. 56, p. 597. 1904.
(5) **Sérégé.** Ibid. 56, p. 600. 1904.
(6) **Sérégé.** Ibid. 58, p. 519. 1905.
(7) **Bartlett, Corper and Long.** Amer. J. Physiol. 35, p. 36. 1914.
(8) **J. J. R. Macleod.** Diabetes. Longmans, Green & Co. New York. 1913.
(9) **Mottram.** This Journal, 38, p. 281. 1909.
(10) **Mottram.** Ibid. 40, p. 122. 1910.
(11) **Rosenfeld.** Ergeb. d. Physiol. 2, 1 abt. 1903.
(12) **Imrie.** J. of Path. and Bact. 19, p. 245. 1915.

# CHROMATIC ABERRATION AND RESOLVING POWER OF THE EYE. BY H. HARTRIDGE, *Fellow of King's College, Cambridge.*

## CONTENTS.

PAGE

1. Hypotheses advanced for non-observance of chromatic error of the eye . . . . . . . . . . . 176
2. Refractive indices, focal lengths, and sizes of aberration discs of the eye for rays of different wave length . . . 179
3. Position of retina in relationship to focal planes of rays of different wave length . . . . . . . . . 181
4. Apparent boundary of fringe system produced by chromatic aberration . . . . . . . . . . . 185
5. Effects of colour mixture in the images produced by a lens uncorrected for chromatic difference of focus . . . 198
6. Effect of certain physical and physiological factors on the appearance of fringes produced by chromatic aberration . 211
7. Chromatic aberration with night vision . . . . . 215
8. Chromatic difference of magnification and chromatic stereoscopy . . . . . . . . . . . . 217
9. Effect of chromatic aberration on the images of coloured objects . . . . . . . . . . . . . 225
10. The diffraction and resolving power of the eye . . . . 229

IT can be shown conclusively by a number of different experimental methods that the optical system of the eye suffers to a considerable extent from chromatic aberration. Various hypotheses have been advanced to explain how it is that in spite of this, the eye does not see coloured fringes at the edges of objects and further how it comes about that the acuity of vision is fully as great as the resolving power of the retina, calculated from the fineness of its structure, would seem to permit. The object of this paper is to criticise these hypotheses and to introduce such modifications as appear necessary in the light of the additional information that is now available.

### 1. HYPOTHESES ADVANCED FOR NON-OBSERVANCE OF CHROMATIC ERROR OF THE EYE.

The effects of chromatic difference of focus are so detrimental to the images formed by optical instruments that there is considerable scope for speculation why the eye should show them to so slight an extent. Several conjectures have been proposed.

*1st hypothesis.* It has been attributed to the tenuity of the luminous rays which pass through the pupil, and to these rays meeting the refractive media of the eye at almost normal incidence (11). This explanation is not justified for, although it is true that when the eye is receiving light of considerable intensity the pupil is small, yet under normal circumstances it is of considerable diameter compared with the focal length of the eye. In ordinary light the diameter of the pupil may be as large as 4 millimetres, which is approximately one-fifth the mean focal length of the eye. Moreover chromatic aberration is not observed with greater facility when the illumination is feeble and the pupil large than it is when the illumination is intense and the pupil small.

*2nd hypothesis.* It has also been suggested that since the media of the eye have approximately the same optical properties as water and since the dispersion of water is small, the focussing points of the different coloured rays lie very little away from one another. Still more is this the case because the total focal length of the eye is short (12). That this explanation is untenable is shown by the following considerations. The factor which determines the amount of chromatic difference of focus present in any case is the ratio of the mean refractive index of the lens medium to its dispersive power. This factor is called "Relative Dispersion." A lens made of a medium with a high index will show a small degree of chromatic aberration, and *vice versa*. The relative dispersion for light crown glass has a value of 70 whereas for dense flint it is 20 approximately (13). The value for water is 54·7 which would correspond to a dense crown glass. The calculations of the probable refractive indices of the eye which will be given later show the value of the relative dispersion to be 45; which would correspond to that of a light flint glass. The amount of chromatic aberration exhibited by the eye is therefore considerable; being very much greater than that which is permissible in optical instruments. But further, the fact that the mean focal length of the eye is short and therefore the chromatic difference of focus for the ends of the spectrum correspondingly small, does not affect the apparent magnitude of the error when the eye is used in vision, because the focal length and the chromatic difference of focus are in constant ratio to one another. This ratio for the eye is about 25 to 1. If for example a lens of 10 inches focal length were constructed from the same formula and from media of the same properties as those of the eye, and if comparison were made between the diameters of the aberration discs formed by this lens and the eye, then it can be shown by calculation that for similar apertures the ratio between the diameters of the aberration discs is the same as that between the focal lengths. It is found however that the dimensions of the images of external objects formed by the two lenses also vary in the same ratio. The visual effect of chromatic aberration on the images of external objects is therefore the same in the two cases.

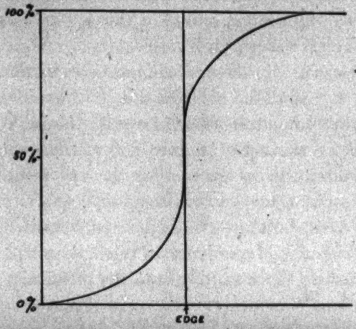


Fig. 1. Intensity curve showing illumination at different points near the edge of an extended source. After Helmholtz. Intensity percentage vertical. Distances from edge of source horizontal.

*Helmholtz' hypothesis.* The most plausible explanation of the non-observance of chromatic aberration that has hitherto been advanced is that proposed by Helmholtz(8). When certain rays near the middle of the spectrum are sharply focussed on the retina, the aberration discs formed by the red and violet rays are of nearly the same diameter and overlap to form an aberration image of relatively small size. He calculated the diameter of the image to be ·0426 mm. when the pupil was 4 mm. diameter. The intensity at different points within the image is, however, far from uniform, for at its centre are concentrated the sharply focussed rays corresponding to the middle of the spectrum. Since these rays are of high visual luminosity the apparent intensity of the centre of the aberration image is very great. **Helmholtz** investigated the distribution of intensity in the fringe caused by chromatic aberration at the edge of an illuminated area. He concluded that the intensity curve has the form shown in Fig. 1.

It will be seen that the intensity decreases suddenly at the point in the fringe which corresponds in position to the geometrical image of the edge of the source. **Helmholtz**, assuming that the eye would recognise this fall in the curve as the true boundary of the fringe, pointed out that under these conditions the eye would see objects their true size undisturbed by the aberration fringe.

Experiments that I have carried out have shown that although Helmholtz' hypothesis is correct in principle still there are a number of features in which it is incomplete. These may be briefly summarised as follows: (1) The refractive indices of the eye are greater than those of water; Helmholtz' values for the diameters of the aberration discs are therefore too small. (2) The rays found by experiment to be sharply focussed on the retina differ considerably in wave length from those that would be expected on the basis of the hypothesis. (3) The intensity curve calculated by **Helmholtz** does not apply to the case of small objects, neither is any proof given that the eye recognises the fall in the centre of the curve as the true boundary of the fringe. (4) The arrangement of colours in the fringe are not considered. Experiment shows that this factor is of considerable importance, particularly in the case of small objects. (5) In addition to the physical and physiological factors considered by **Helmholtz** there are others which are important since they have the effect of reducing the apparent magnitude of the aberration image. (6) The hypothesis did not take into account the special conditions that occur in night vision. (7) **Helmholtz** did not consider the effects of chromatic difference of magnification. These, as **Gullstrand** has pointed out, follow as a direct development from Helmholtz' theory.

I find that there is another explanation for the non-observance of chromatic difference of magnification in addition to that advanced by **Gullstrand**. The following sections of this paper give a brief description of these additional features.

### 2. REFRACTIVE INDICES, FOCAL LENGTHS AND SIZES OF ABERRATION DISCS OF THE EYE FOR RAYS OF DIFFERENT WAVE LENGTH.

**Helmholtz**(8) calculated the diameter of the aberration discs on the assumption that the refractive indices of the eye are equal to those of water, namely, 1·331705 for the *C* line in the red end of the spectrum and 1·341285 for the *G* line in the blue end of the spectrum. He found that red light would be focussed 20·574 mm. and blue light 20·141 mm. from the surface of the eye. The difference of focus according to **Helmholtz** would therefore be ·434 mm. That the values thus obtained are too low is shown by the experimental determinations of **Wolf**, **Einthoven**, and others. Thus **Fraunhofer**(6) by measuring the distance at which he could clearly focus a strand of spider's web illuminated by light corresponding to chosen parts of the spectrum estimated the difference of focal length to be ·47–·63 mm. **Matthiessen**(7) using monochromatic filters obtained a higher value, namely, ·58–·62 mm. Experimental determinations by **Wolf**(9) show a higher value. He found the difference in focal length to be ·75 mm. for rays corresponding to the *B* and *H* Fraunhofer lines, and ·5 mm. for the *C* and *G* lines. **Einthoven**(10) who has recently calculated from Kunst's figures the cardinal points of the eye for different parts of the spectrum also finds Helmholtz' values too low. He estimated the difference of focal length for the *D* and *F* lines to be ·272 mm.; he found, however, the posterior focal plane to be practically constant in position for all colours. The method by which the approximate values of the refractive indices can be obtained is as follows. **Tscherning**(14) showed that the eye can be regarded as consisting of two refracting systems; the one having the same refractive index as water, and forming the globe of the eye; the other representing the crystalline lens and having a mean refractive index of 1·42. The refracting powers of these systems he found to be 42 and 16 diopters respectively. The mean refractive index of the eye as a whole can be obtained approximately by taking the mean between the refractive indices of the systems multiplied by their respective powers in diopters. In this way the value 1·387 is obtained as the mean refractive index of the eye for rays corresponding approximately to the *D* Fraunhofer line in wave length (5893 A.U.). The posterior focal length of the eye for the same rays is 20·5 mm. **Wolf's** figures(9) for the chromatic difference of focus for rays corresponding in wave length to the *B* and *H* lines is ·75 mm. and for the *C* and *G* lines ·5 mm. Assuming that the change in refractive index with wave length is in the same ratio as that of water and that the values are such that the difference between the focal lengths of the eye are the same as those found by Wolf, the following values were obtained for the refractive indices and corresponding focal lengths of the eye.

TABLE 1. Values of refractive indices and focal lengths of eye for rays of different wave length.

| Fraunhofer line | *B* | *C* | *D* | *E* | *F* | *G* | *H* |
|---|---|---|---|---|---|---|---|
| Wave-length in A.U. | 6867 | 6563 | 5893 | 5270 | 4862 | 4308 | 3969 |
| Refractive index | 1·3830 | 1·3840 | 1·3870 | 1·3900 | 1·3930 | 1·3975 | 1·4040 |
| Focal length mm. | 20·67 | 20·62 | 20·53 | 20·42 | 20·30 | 20·12 | 19·92 |

These values for the chromatic difference of focus of the eye will be seen to fit in approximately with Wolf's experimental values. The difference for *D* and *F* namely, ·23 mm., is, however, considerably less than that given by Einthoven (·2718 mm.); and it is impossible to obtain values which fit in both with those of Einthoven and Wolf. Until this difference is decided I have preferred to adhere to Wolf's values since I believe them to be nearer the truth. According to the above table, therefore, the relative dispersion of the eye has a value of 45. Since the value of this ratio for water is 54·7 the chromatic difference of focus of the eye is approximately 21·5 % greater than it would be if the eye media had the same refractive index as water. This difference is therefore important. (According to Einthoven's figures the relative dispersion would equal 38; which would cause the eye to have a chromatic difference of focus 44 % greater than water.) From the refractive indices given in the above table the diameters of the aberration discs were calculated by means of the following formula:

$$A = \frac{P}{\mu Y - 1} \times \frac{\mu Y - \mu X}{\mu X} \quad \dots\dots\dots\dots\dots\dots\dots\dots (1).$$

Where $A$ is diameter of aberration disc, $P$ the diameter of the pupil, $\mu Y$ the refractive index of the eye media for rays focussed on the retina and $\mu X$ that for rays forming the aberration discs.

It will be observed that according to the above formula the diameter of the disc is independent of the focal length and depends solely on the optical constants of the lens medium and the diameter of the pupil. Further that in Table 2 the diameters of the aberration discs are given for 4900 A.U. and 5800 A.U. It will be shown in the next section that the former is the wave length of the rays which according to Helmholtz are those which are focussed sharply on the retina under normal circumstances, whereas the latter is the wave length found experimentally by the author.

**Table 2.** Mean refractive indices of eye for light of different wave length, and diameters of aberration discs on the Author's and Helmholtz' basis, assuming the pupil to be 1 mm. diameter.

| Wave length ... | 7500 | 7000 | 6500 | 6000 | 5500 | 5000 | 4500 | 4000 |
|---|---|---|---|---|---|---|---|---|
| Refractive index | 1·3810 | 1·3826 | 1·3844 | 1·3864 | 1·3888 | 1·3920 | 1·3960 | 1·4025 |
| Diameter of disc for 5800 A.U.... ... | ·0121 | ·0092 | ·0058 | ·0021 | ·0024 | ·00815 | ·0157 | ·0276 |
| Diameter of disc for 4900 A.U.... ... | ·0190 | ·0178 | ·0145 | ·0108 | ·0064 | ·0008 | ·0068 | ·0169 |

It will be noted that the largest aberration disc is found on the author's basis, namely, ·0276 mm. for a ray of 4000 A.U. wave length, the diameter of the aberration disc formed by the rays from the other end of the spectrum being less than one-half this value. Whereas on Helmholtz' basis both discs are nearly equal in size and are of an intermediate diameter. The diameters of the aberration discs are shown plotted in Fig. 2.

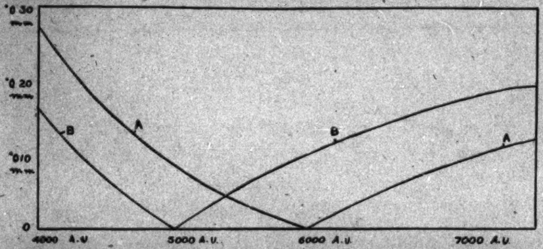


Fig. 2. Diameters of aberration discs formed by rays of different wave length. (Pupi 1 mm. diameter.) *A.* Author's basis. *B.* Helmholtz' basis. Wave length in A.U. vertical. Diameter in millimetres horizontal.

### 3. POSITION OF RETINA IN RELATIONSHIP TO FOCAL PLANES OF RAYS OF DIFFERENT WAVE LENGTH.

Helmholtz' hypothesis can be shown to be at fault for several reasons. (1) Experiment shows that when white objects are focussed on the retina yellow and not green rays form the most sharply focussed images. (2) Definite advantages would appear to follow the focussing of yellow rays in preference to green. (3) Calculations based on the diameters of the aberration discs produced by rays of different wave length confirm the hypothesis that advantages follow the focussing of yellow rays. (4) Experiments with a telescope fitted with an uncolour-corrected objective give evidence that the above conclusions are true.

The retina on Helmholtz' hypothesis receives the sharply focussed images formed by rays which come from near the centre of the spectrum. These are of such a wave length that the aberration discs formed by the rays from the ends of the spectrum are of equal diameter. Their wave length can be calculated in the following manner: In Fig. 3, $B1$, $B2$ are the edges of the pupil, $FV$ is the focal distance for violet rays, $FR$ that for red rays. The aberration discs formed by the red and violet rays are of equal diameter at the plane $CC'$. The retina, according to Helmholtz, is situated in this plane. $FD$ is therefore the focal distance of the rays which form sharply focussed images on the retina.

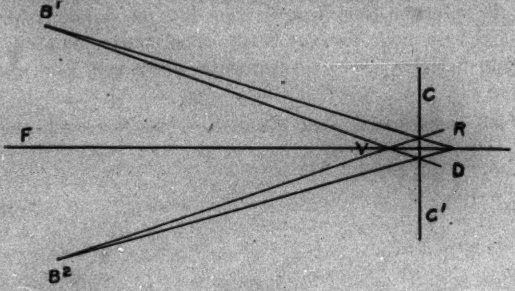


Fig. 3.

From similar triangles

$$DV \times FR = DR \times FV.$$

Also $DV = FD - FV$ and $DR = FR - FD$.

Therefore by substitution and multiplying both sides of the equation by $FR \times FV$, we find that $(FD - FV)\,FR = (FR - FD)\,FV$.

Therefore $$FD\,(FR + FV) = 2 \times FR \times FV \quad \text{.....................} (2).$$

Inserting Helmholtz' values for $FR = 20{\cdot}574$ and $FV = 20{\cdot}141$, we find that

$$FD = 20{\cdot}355.$$

The refractive index of the ray with this focal distance is given by the formula

$$FD = \mu\,(FD - R) \quad \text{.....................} (3),$$

where $R$ is the radius of the cornea (5·1248 mm.). This gives 1·3365 as the value for the refractive index. Now this is the index of water for a ray of wave length 5120 A.U. which is in the green region of the spectrum.

(From my values of the refractive indices of the eye which are given in the previous section, a somewhat smaller value, namely 4950 A.U. was obtained.)

The experimental evidence was obtained as follows:

Pieces of unspun silk were supported in a frame so that they formed a series of parallel strands. Behind the frame was mounted at an angle of 45° a silvered mirror from which strips of the silver had been removed. This was done in such a way that alternate strands of the silk could be illuminated by transmitted and reflected light. Two uniform artificial light sources were mounted in the required positions. A series of light filters each of which isolated a narrow spectral area was placed in turn in front of one of the lights. In front of the other being placed colourless absorbing media so as to make the reflected and transmitted beams nearly equal in intensity. It was found with this apparatus that when the light filter transmitted a band corresponding to either end of the spectrum, it was impossible to focus the alternate sets of strands simultaneously. When a yellow light was used, however, both sets appeared sharp, the strands illuminated by the yellow light being if anything more clearly defined than those illuminated by the white. The adjustment is not a sharp one, however, a considerable change in the position in the spectrum of the band transmitted by the filter did not obviously affect the sharpness of the lines.

The mean wave length of the light shown by this method to most closely correspond in its focal plane to that of white light was found to be approximately 5800 A.U. This is the wave length of a ray in the yellow region of the spectrum, nearly 800 A.U. longer than the value calculated above on Hemholtz' basis. This will cause the violet aberration circle to be larger, and that of the red end of the spectrum correspondingly smaller. The probable reason for this selection by the eye of yellow rays as the focussing point in preference to green rays, is the greater luminosity of the yellow region of the spectrum.

The actual wave length of the ray chosen would therefore be found to vary with the following factors:

(1) The intensity of the image formed on the retina. In accordance with Purkinji's phenomenon the wave length will be longer at high intensities and smaller at low. With night vision it will be found to be near 5350 A.U. The effect of intensity will be referred to again in the sixth section of this paper.

(2) The spectro-photometric composition of the rays proceeding from the light source. The wave length will be longer when the light source is rich in red rays, by lamplight for example, and shorter for a source rich in violet rays such as daylight.

(3) The effort required for accommodation is in normal vision greater

for red rays than for violet. The retina should, therefore, normally correspond to rays of slightly shorter wave length than the optimum. When the eye is astigmatic the retina, as is well known, may correspond with the focal point of red rays in one axis and of violet in the axis at right angles. A delicate test for astigmatism has been made on this basis; it will be found described at the end of the ninth section of this paper.

The selection of yellow rays as the focussing point in preference to green rays has the following advantages:

(1) The yellow rays forming the centre of the fringe have a luminosity greater than any other part of the spectrum and nearly four times that of the green (5000 A.U.). The sharpest images are therefore formed by rays of the greatest intensity.

(2) The aberration discs formed by the red, orange and green rays, which have nearly half the intensity of the yellow rays, are of small diameter and therefore contribute to the brightness of the image without degrading the definition.

(3) The blue and violet rays, which are visually of the lowest luminosity, approximately one-twentieth that of the yellow, form diffusion circles of greater diameter when yellow rays are selected to form the centre of the fringe in preference to green rays. The poor definition of the images which they form is therefore rendered less noticeable, owing to the larger area over which their intensity is spread.

These claims of advantage for yellow rays as the focussing point are confirmed by the calculations of the intensity distributed over the fringe which will be given in the next section of this paper. The advantages given by the selection of the focal plane for yellow rays can be demonstrated experimentally by means of a telescope in which the object glass has been replaced by an uncorrected crown glass lens of similar focal length. When the telescope is focussed in daylight at the focal plane for yellow rays the image is sharp and the coloured fringes unobtrusive. When apple green rays are focussed the image is apparently equally sharp but the coloured fringes are slightly more noticeable than in the previous case.

When the telescope is focussed for blue green rays the definition is poor and the coloured fringes are found to be very strongly developed. When an artificial light is used, particularly when the light is yellow in colour, such as that from an ordinary paraffin oil lamp the best focussing point appears to be nearer the orange part of the spectrum.

### 4. APPARENT BOUNDARY OF FRINGE SYSTEM PRODUCED BY CHROMATIC ABERRATION.

#### *Source of considerable dimensions.*

It will be shown in the two following sections that chromatic aberration modifies the images formed on the retina principally in two ways. It diminishes their sharpness, and it causes certain colour effects to be developed. The effects on definition will be considered in this section.

Helmholtz calculated that in the case when the source is of considerable dimensions the intensity curve showed a very rapid change where the fringe corresponded in diameter with the geometrical image of the source. On the supposition that the eye recognises this sudden change of intensity as the true boundary of the fringe, he pointed out that objects will appear their true size. Helmholtz did not obtain, however, any experimental proof that such in fact was the case, neither did he consider the form and apparent size of the fringe in the case where the object is small. With regard to the first question I have investigated experimentally the apparent edge of the fringe in the case of large sources by means of the rotating disc method used in a previous research (15). On a circular disc of white card were drawn a series of black areas the shapes of which corresponded with the form of the intensity curve calculated by Helmholtz. The disc was now rotated electrically at a high speed so that the areas were spread out to produce the fringe. The position of the apparent edge of the image was measured with dividers, care being taken to eliminate parallax. In the case where the disc had a white centre it was found that the edge was slightly nearer the periphery of the disc than was the position of the fall in the intensity curve. Experiment showed, therefore, that Helmholtz' supposition was correct, the slight increase in diameter as measured by dividers being explained either by the presence of irradiation, by slight eccentricity of the disc, or by experimental error. To a close approximation, therefore, large objects are seen their true size.

#### *Point source.*

Considerable difficulty exists in the determination of the intensity distribution in the images of the small objects formed on the retina, because of the complexity and number of the factors which come into play. Apart from chromatic difference of focus which is normally present in the eye, other aberrations are frequently present to a greater or less extent; spherical aberration may be mentioned as an example. But apart from errors in the refracting media of the eye there is a further factor which comes into operation because of the undulatory character of light, namely, diffraction by the pupil of the eye. This causes the image of a point source to take the form of a bright spot surrounded by a series of luminous rings. In the image of the normal eye these effects are superimposed on one another to form an exceedingly complex picture. It will be my purpose in § 10 to consider this formation of the image as fully as possible, particularly in its relationship to the resolving power of the eye. For the present purpose I therefore propose to consider the effects of chromatic aberration alone, excluding the other aberrations and diffraction.

At the end of § 1 of this paper I have given the calculated values for the diameters of the aberration discs formed by rays of different wave length in the case of a pupil of 1 mm. in diameter. It will be seen that discs smaller in diameter than ·012 mm. are formed by rays coming from both red and green sides of the yellow in the spectrum, whereas larger discs than this are formed by rays of short wave length only.

The wave lengths of the rays forming a disc of any given size can be at once obtained by reference to curve *A* in Fig. 2. Now the physiological luminosity of different regions of the spectrum have been investigated by Haycraft(26) and others. Probably the determinations carried out by Abney on the spectrum of the electric arc are the most accurate available(16). He found that the yellow region was the most luminous, the values decreasing on either side of this maximum. The luminosity curve strongly resembles that of a variation obeying Maxwell's law of chance. (Houston(17).)

In Table 3 are given the wave lengths and luminosities of the rays forming the aberration discs of different diameter. The figures in the 6th column show the combined luminosity of the rays of both longer and shorter wave length than the yellow. The luminosity is, however, spread over an area which varies with the square of the diameter of the disc. The value of the intensity of the disc is therefore obtained by dividing the luminosity by the area. In the case of the sharply focussed yellow rays the area covered by the image, formed by a lens that does not suffer from the effects of other aberrations and diffraction is infinitely small. The intensity at the centre of the image therefore rises to infinity. The other values of the intensity for discs of different diameter are given in col. 7. It will be observed that the values fall from infinity exceedingly rapidly, becoming negligible for discs of less than half the maximum diameter.

TABLE 3. Total luminosity and intensity of aberration discs of different diameter (on Author's basis). (The pupil diameter is assumed to be 1 mm.)

| Diameter of disc in mm. | Long Component | | Short Component | | Total luminosity | Intensity of disc |
|---|---|---|---|---|---|---|
| | Wave length | Luminosity | Wave length | Luminosity | | |
| 0 | 5800 | 98 | 5800 | 98 | 196 | ∞ |
| ·001 | 5900 | 100 | 5680 | 90 | 190 | 242 |
| ·002 | 6000 | 100 | 5560 | 76 | 176 | 56 |
| ·003 | 6120 | 91 | 5460 | 64 | 155 | 21·3 |
| ·004 | 6270 | 72 | 5350 | 52 | 124 | 9·9 |
| ·005 | 6400 | 48 | 5280 | 42 | 89 | 4·5 |
| ·006 | 6550 | 22 | 5150 | 32 | 54 | 1·9 |
| ·007 | 6700 | 8 | 5100 | 20 | 28 | ·73 |
| ·008 | 6850 | 4 | 5020 | 11 | 15 | ·30 |
| ·009 | 7000 | 1·5 | 4940 | 7·5 | 9 | ·14 |
| ·010 | 7150 | 0 | 4870 | 5·0 | 5 | ·06 |
| ·011 | 7300 | 0 | 4800 | 3·2 | 3·2 | ·04 |
| ·012 | 7500 | 0 | 4740 | 2·3 | 2·3 | ·02 |
| ·014 | — | — | 4620 | 1·7 | 1·7 | ·01 |
| ·028 | — | — | 3970 | 0 | 0 | 0 |

Table 3 is calculated on the assumption that the most sharply focussed rays are yellow (5800 A.U.). If Helmholtz' value is used (4900 A.U.) then the following values are obtained.

TABLE 4. Total luminosity and intensity of aberration discs of different diameter (on Helmholtz' basis). (The pupil diameter is assumed to be 1 mm.)

| Diameter of disc in mm. | Long Component | | Short Component | | Total luminosity | Intensity of disc |
|---|---|---|---|---|---|---|
| | Wave length | Luminosity | Wave length | Luminosity | | |
| 0 | 4900 | 6·3 | 4900 | 6·3 | 12·6 | ∞ |
| ·001 | 4980 | 8·8 | 4850 | 4 | 12·8 | 16·3 |
| ·002 | 5060 | 16 | 4790 | 3·2 | 19·2 | 6·1 |
| ·003 | 5140 | 24 | 4720 | 2·2 | 26·2 | 3·7 |
| ·004 | 5240 | 35 | 4670 | 2·0 | 37·1 | 3 |
| ·005 | 5350 | 49 | 4610 | 1·5 | 50·5 | 2·6 |
| ·006 | 5470 | 64 | 4550 | 1·2 | 63·2 | 2·3 |
| ·007 | 5570 | 76 | 4480 | 1·0 | 77·0 | 2 |
| ·008 | 5680 | 86 | 4420 | ·8 | 86·8 | 1·7 |
| ·009 | 5790 | 96 | 4360 | ·6 | 96·6 | 1·6 |
| ·010 | 5910 | 99 | 4310 | ·5 | 99·5 | 1·3 |
| ·011 | 6030 | 100 | 4260 | ·4 | 100·4 | 1·1 |
| ·012 | 6170 | 88 | 4210 | ·3 | 88·3 | ·8 |
| ·014 | 6410 | 34 | 4125 | ·2 | 34·2 | ·2 |
| ·028 | — | 0 | 3970 | 0 | 0 | 0 |

If the intensity values of the discs given in Tables 3 and 4 be compared it will be seen that those of smaller diameter than ·0055 mm. are brighter on the author's basis than they are on Hemholtz'; whereas with those of larger diameter than ·0055 mm. the reverse is the case. These differences are well shown by the curves in Fig. 4.

These figures therefore show that a greater percentage of the light forms the sharply focussed picture on the author's basis than it does on that of Helmholtz. The deductions made at the end of the last section are therefore substantiated.

From the figures for the intensity of aberration discs of different diameter shown in the table above the intensity at different points of the image in the case of a point source can be obtained by summation.

Since the intensity of the aberration discs follows no mathematical law it is impossible to employ any process of integration. The intensity values of the image were therefore calculated by adding together the intensities of the different discs which illuminate any given point. The figures thus obtained are not exact since they assume the variation of intensity with diameter to remain constant over the range between two discs. By making this interval small figures approximating closely to the true intensity values are obtained. No advantage is obtained by giving the values to more than three significant figures, since variations in the quality of the light and the intensity of illumination will cause far larger changes under conditions met with in practice.

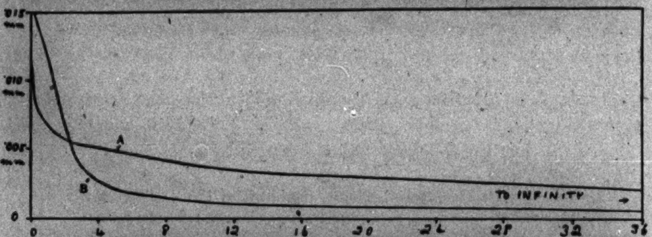


Fig. 4. Intensity of illumination of aberration discs of different diameter. (Pupil 1 mm.) *A*. Author's basis. *B*. Helmholtz' basis. Intensity of illumination vertical. Diameter of image in mm. horizontal.

The curve obtained by plotting the intensity values in Table 5 is shown in Fig. 5 (*A*). The curve has the same general characteristics as the intensity curve of the individual aberration discs, which is shown in Fig. 4 (*A*). The intensity at the centre of the image will only reach infinity under ideal conditions, that is, where the object is a mathematical point of light and where no diffraction occurs at the edge of the pupil, the refracting system of the eye being perfect in every way with the single exception of chromatic aberration. Owing to the special features of the intensity curve mentioned above, the distribution of light in the image is very favourable. Thus it is found that over 76 % of the light falls within a circle of one-tenth the diameter of the maximum aberration disc. With a pupil of ·9 mm. diameter this circle will have a diameter of ·0025 mm. Now this corresponds approximately with the probable

TABLE 5. Intensity at different points of the image of a point source. (Distances from centre are given as decimals of maximum radius of image.)

| Distance from centre | Intensity of image | Distance from centre | Intensity of image |
|---|---|---|---|
| 0 | ∞ | ·240 | 2·2 |
| ·009 | 1491 | ·259 | 1·46 |
| ·027 | 501 | ·277 | ·99 |
| ·045 | 259 | ·295 | ·69 |
| ·063 | 155 | ·313 | ·50 |
| ·080 | 99 | ·331 | ·37 |
| ·098 | 65 | ·347 | ·26 |
| ·116 | 43·7 | ·366 | ·21 |
| ·134 | 29·2 | ·383 | ·16 |
| ·153 | 19·3 | ·401 | ·11 |
| ·170 | 12·7 | ·419 | ·09 |
| ·188 | 8·2 | etc. | — |
| ·206 | 5·3 | 1·0 | 0 |
| ·223 | 3·4 | | |

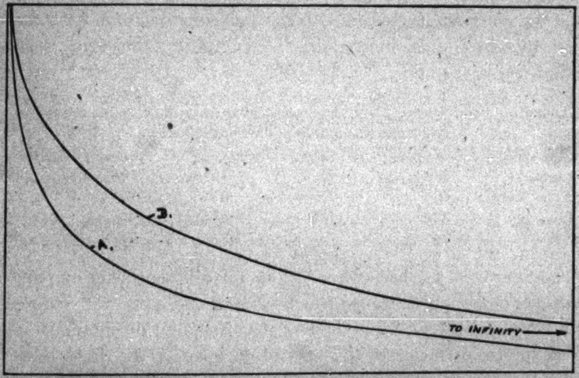


Fig. 5. Comparison between curves of intensity of illumination for point and linear sources. Curve *A*, point source; Curve *B*, linear source. Vertical intensity of illumination. Horizontal distance from centre of source in mms.

diameter of the retinal cone elements of the fovea (values ranging between ·002 and ·0054 mm. have been given by different observers (18)).

It will thus be seen that when the pupil is ·9 mm. diameter over 76 % of the light is concentrated on a single cone. Calculation shows that the cones neighbouring this each receive approximately 3 % of the light. It is well known that letters which appear black to the eye, actually reflect from 5 to 10 % of the light that comes from the white surface on which they are printed; the blackness of the images is found to be largely due to the fact that the intensity difference is greatly enhanced by contrast. It can be assumed that a similar effect will take place in that area of the retina which is illuminated by the chromatic image. Contrast will, therefore, cause the cones on which only 3 % of the light falls to remain unresponsive; and one cone alone will respond to the stimulus, namely, that on which the centre of the image falls.

This shows that when the pupil is ·9 mm. in diameter or smaller the effects of chromatic aberration are negligible, so far as the stimulus received by the cone elements of the retina are concerned.

The intensity of the light falling on the retina has been calculated in a similar manner for other diameters of pupil. The results are shown in Table 6 A, the first ring of cones being those that surround the cone on which the centre of the image falls, the second ring those that surround the first ring and so on.

TABLE 6 A. Point source. Showing percentage intensity illuminating retinal cone elements with pupils of different diameter.

| Pupil diameter | Central cone | 1st ring | 2nd ring | 3rd ring | Cones width |
|---|---|---|---|---|---|
| ·9 mm. | 76·4 % | 3 % | ·18 % | — | 10 |
| ·2 ,, | 46 | 5·5 | ·9 | ·07 % | 22 |
| ·4 ,, | 32 | 5·1 | 1·55 | ·45 | 44 |
| ·6 ,, | 30 | 5·1 | 1·8 | ·7 | 66 |

The figures in the last column show for different sizes of pupil the number of cones which placed side by side would equal the diameter of the aberration image. Thus in the case of a pupil of ·9 mm. diameter the maximum diameter of the aberration image is 10 times that of a cone, and yet as has been shown above the intensity is so distributed that chromatic aberration does not spoil the image. Such a state of affairs would appear remarkable. The figures for other diameters of pupil are no less interesting. Thus with a pupil of 2 mm. nearly half of the light which falls on the retina is concentrated on to the central cone, in spite of the fact that the aberration image is 22 cones wide and nearly 500 cones in area. The illumination of the 1st ring of cones is relatively greater than in the previous case, being 12 % of that falling on the central cone.

This amount of light would if diffusely reflected from a surface of appreciable size appear a very dark grey to the eye, and would probably not detract from the sharpness of the image, to a very noticeable extent. Pupils of 4 mm. and over are, as a rule, only found with low illumination and it would be exceptional to find a pupil larger than 6 mm. used at any other time than at night. Under these conditions, as is well known, the power to discriminate between grey and black is much impaired; a surface reflecting diffusely 20 % of the incident light appearing the same to the eye as a surface reflecting almost none. It is, therefore, not difficult to suppose that under these circumstances the images presented to the eye appear sharp, even with such large pupils.

With night vision, however, additional factors come into play which will be shown in § 6 to diminish the effects of chromatic aberration.

### *Linear source.*

The intensity of illumination at different points of the image of a linear source can be calculated from the intensity values of the discs of different diameter for a point source, since a linear source may be regarded as an infinite number of point sources placed side by side in a straight line. One would expect this overlapping of the discs to cause the rate of fall of intensity as the distance from the centre of the image increases to be less rapid than is the case with a point source. The values given in the table and the figures plotted from them show that this supposition is correct.

**TABLE 6 B.** Comparison between intensity value at different points of the images of a point and linear point source. (Distances from the centre are given as decimals of maximum radius of image, namely, ·014 mm. with a pupil of 1 mm. diameter.)

| Distance from centre | Intensity Linear source | Intensity Point source |
|---|---|---|
| 0 | ∞ | ∞ |
| ·009 | 1490 | 1490 |
| ·027 | 833 | 501 |
| ·045 | 547 | 259 |
| ·063 | 329 | 155 |
| ·080 | 269 | 99 |
| ·098 | 189 | 65 |
| ·116 | 131 | 43·7 |
| ·134 | 88·6 | 29·2 |
| ·152 | 57·6 | 19·3 |
| ·170 | 26·7 | 12·7 |

The above values are shown plotted in Fig. 5. Curve *A* being for a point source, Curve *B* for a linear source. If corresponding values for the intensity at different points of the image are compared it will be observed that in every case but the first two those for a point source are lower. The image is, therefore, sharper for a given diameter of pupil in the case of the point source. The same difference is shown when the percentage intensity stimulating the retinal cone elements in the two cases are contrasted (see Table 7 A).

TABLE 7 A. Intensity of illumination falling on cones of retina values for point and linear source compared. (Pupil diameter, 2 mm.)

| | Central cone | 1st row | 2nd row | 3rd row |
|---|---|---|---|---|
| Linear source | 100 | 21·3 | 3·1 | ·45 |
| | Central cone | 1st ring | 2nd ring | 3rd ring |
| Point source | 100 | 12·2 | 1·9 | ·16 |

The less beneficial distribution of intensity in the image of the linear source is compensated by the greater amount of light that is concentrated on the central row of cones, compared with that found in the case of the point source. This is shown in Table 7 B.

TABLE 7 B. Percentage intensity of illumination falling on cone retinal elements in case of linear and point sources. (Pupil diameter, 2 mm.)

| | Central cone | 1st row | 2nd row | 3rd row |
|---|---|---|---|---|
| Linear source | 65·6 | 14·4 | 2·7 | ·9 |
| | Central cone | 1st ring | 2nd ring | 3rd ring |
| Point source | 45 | 5·5 | ·9 | ·07 |

A further fact in the favour of the linear source is the greater amount of white light produced by colour mixture in the image of the linear source than in that of the point source. This will be again referred to in § 5.

### *Sources of finite size.*

The intensity curves given above for the point and linear sources have been developed by different methods from those used by Helmholtz for investigating the intensity at the edge of a large source of light. Further, the intensity curves for the former do not show any direct connection with that of the latter. For this reason the investigation of the intensity for finite sources is of considerable interest since they occupy an intermediate position.

Two types will be briefly considered; the circular source, and the rectangular source. The former being developed from the intensity curve of a point source, the latter from that of the linear source. The calculation in both cases is a simple one since the intensity illuminating the cone elements has already been ascertained for a single point source; (a circular source being regarded as formed from a series of isolated point sources; and the rectangular source similarly considered as composed of a number of parallel linear sources).

TABLE 8. Percentage intensity of illumination falling on cone retinal elements. (Pupil diameter, 2 mm.)

| Central cone | 1st ring | 2nd ring | 3rd ring | 4th ring | 5th ring |
|---|---|---|---|---|---|
| Circular source forming image ·0075 mm. diameter | | | | | |
| 78 | 64·2 | 8·4 | 1·1 | ·08 | ·01 |
| Rectangular source forming image ·0075 mm. in width | | | | | |
| **Central cone** | **1st cone** | **2nd cone** | **3rd cone** | **4th cone** | **5th cone** |
| 95·3 | 83·0 | 16·8 | 2·4 | ·3 | ·09 |

The figures bring out several points of interest. The image according to geometrical optics should stimulate the central cone and one row or ring of cones on either side. There should therefore be a drop from 100 % to 0 in the intensity between the 1st and 2nd row or ring of cones. It will be seen that a considerable fall in the intensity takes place, namely, 67·2 % in the case of the rectangular source, and 55·8 % for the circular source. There is therefore even in sources of such small magnitude the development of a definite "edge" which should be visible to the eye.

There is in these figures the same difference between circular and

TABLE 9. Percentage intensity of illumination at different distances from edge of image of rectangular source ·0075 mm. diameter. (Pupil diameter, 2 mm.)

| Distance from edge (in mm.) | % intensity | |
|---|---|---|
| +·00475 | — | |
| +·00425 | ·4 | Outside image |
| +·00375 | 1·1 | |
| +·00325 | 2·3 | |
| +·00275 | 3·9 | |
| +·00225 | 6·2 | |
| +·00175 | 9·6 | |
| +·00125 | 13·9 | |
| +·00075 | 20·8 | |
| +·00025 | 31·2 | |
| ± 0 | 50·0 | Edge of image |
| −·00025 | 68·8 | Inside image |
| −·00075 | 77·2 | |
| −·00125 | 86·1 | |
| −·00175 | 90·3 | |
| −·00225 | 93·7 | |
| −·00275 | 96·1 | |
| −·00325 | 97·4 | |
| −·00375 | 97·8 | Centre of image |

rectangular sources as have been indicated above in the case of point and linear sources, namely, a more rapid fall in the intensity in the case of the circular source which is partly compensated by the greater values that the intensity reaches in the case of the rectangular source.

The figures in which the average intensity falling on the cones is considered do not show the intensity about the edge of the image so well as values obtained for closer intervals such as are given in Table 9.

These values are shown plotted in Fig. 6 in full lines. The interrupted line shows the average intensity stimulating the cones of the retina. The dotted lines show the intensity distribution in the case of a perfect lens. If the curve in full lines in Fig. 6 be compared with Helmholtz' curve in Fig. 1 and that shown in Fig. 5 in the case of a linear source it will be seen how the transition occurs from one to the other. As the linear source increases in width, the intensity at the centre of the image becomes finite; then the intensity curve develops a sudden increase where it coincides with the edge of the image. The top of the curve continues to rise as the image increases in width but with decreasing speed until finally it reaches a maximum value; this occurs when the width of the image is equal to the diameter of the largest aberration disc. This series of changes is shown in Fig. 7.

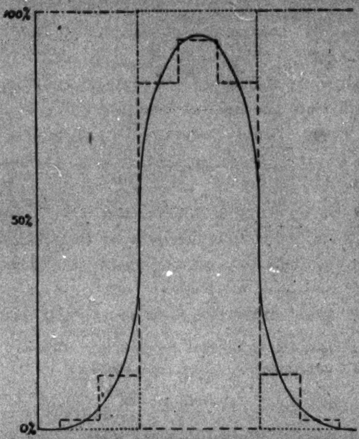


Fig. 6. Curve *A* (full lines), intensity of image. Curve *B* (interrupted lines), average intensity of light incident on cones of retina. Curve *C* (dotted lines), intensity of image formed by ideal lens.

### *Edge of extended source.*

The development of the curve for the edge of an extended source has already been described in the previous sub-section. The intensity values obtained in a similar manner to those previously given in Table 9 are shown below.

TABLE 10. Percentage intensity of illumination at different distances from edge of extended source.

| Distance from edge (in mm.) | % intensity | |
|---|---|---|
| + ·00475 | 0 | Outside image |
| + ·00425 | ·4 | |
| + ·00375 | 1·1 | |
| + ·00325 | 2·3 | |
| + ·00275 | 3·9 | |
| + ·00225 | 6·2 | |
| + ·00175 | 9·6 | |
| + ·00125 | 13·9 | |
| + ·00075 | 20·8 | |
| + ·00025 | 31·2 | |
| ± 0 | 50·0 | Edge of image |
| − ·00025 | 68·8 | Inside image |
| − ·00075 | 77·2 | |
| − ·00125 | 86·1 | |
| − ·00175 | 90·3 | |
| − ·00225 | 93·7 | |
| − ·00275 | 96·1 | |
| − ·00325 | 97·7 | |
| − ·00375 | 98·9 | |
| − ·00425 | 99·6 | |
| − ·00475 | 100 | |

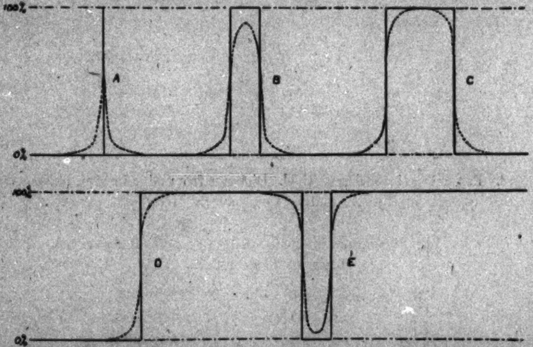


Fig. 7. Curve *A*, linear source. Curve *B*, source ·0075 mm. width. Curve *C*, source ·018 mm. wide. Curve *D*, edge of extended source. Curve *E*, interval between two sources ·0075 mm. (Pupil 2 mm. diameter.)

In Fig. 8 the full line shows the curve obtained by plotting the above figures; the dotted line shows Helmholtz' curve for comparison. The similarity between the two curves is well shown. In both, the greatest rate of change of intensity corresponds with the geometrical image of the edge of the source. The fact that the two curves have been arrived at by entirely different methods is of interest. Additional weight is therefore given to his suggestion that objects must under these conditions appear their true size. The principal difference between the curves is the sharper fall in intensity found by the author. This is probably due to the fact that Helmholtz had to make the assumption that the intensity throughout the spectrum was uniform whereas in the author's curve the different luminosity values of the colours has been taken into consideration.

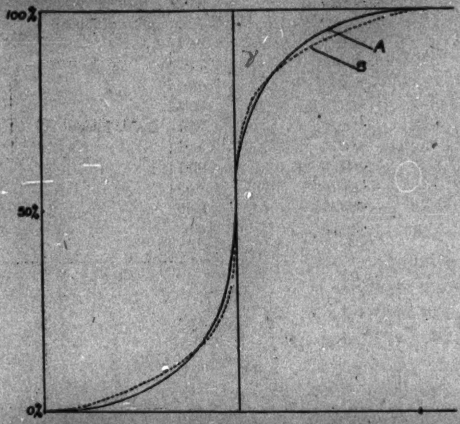


**Fig. 8.** Percentage intensity vertical. Distance from edge in mm. horizontal. (2 mm. pupil.) *A.* Author's curve. *B.* Helmholtz' curve.

**Table 11.** Percentage intensity of illumination falling on cone elements of the retina.

| Rows of cones | % intensity | |
|---|---|---|
| 1st ... ... ... | 0 | Outside image |
| 2nd ... ... ... | ·3 | |
| 3rd ... ... ... | 2·4 | |
| 4th ... ... ... | 16·8 | |
| Edge of image | | |
| 5th ... ... ... | 83·2 | Inside image |
| 6th ... ... ... | 97·6 | |
| 7th ... ... ... | 99·7 | |
| 8th ... ... ... | 100 | |

These values are plotted as broken lines in Fig. 8.

The intensity stimulating the different rows of cones are found to be as above (Table 11).

The figures show that the difference in intensity stimulating the cones on either side of the edge of the image is 60·4 %.

### *Interval between two extended sources.*

This is the case of a black object lying on an illuminated ground. It is found that there are two methods by which the intensity can be calculated. Firstly, by regarding each source separately and summing the intensity values where they are found to overlap. Secondly, by imagining the interval formed by removing a strip from a uniformly illuminated source of width equal to that of the interval. According to the second method the intensity values are the complement to those of a bright source (of equal width with the interval placed on a dark ground). Thus suppose the width of the image of the interval to be equal to three times one cone diameter the intensity values may be obtained by taking complementary figures to those given in Table 9 above. In this way the following values were obtained.

TABLE 12. Percentage intensity at different points of aberration image of interval between two extended sources ·0075 mm. width. (Pupil diameter, 2 mm.)

| Distance from edge of image in mm. | % intensity | |
|---|---|---|
| + ·00475 | 100 | Outside image |
| + ·00425 | 99·6 | |
| + ·00375 | 98·9 | |
| + ·00325 | 97·7 | |
| + ·00275 | 96·1 | |
| + ·00225 | 93·7 | |
| + ·00175 | 90·3 | |
| + ·00125 | 86·1 | |
| + ·00075 | 79·2 | |
| + ·00025 | 68·8 | |
| ± 0 | 50·0 | Edge of image |
| − ·00025 | 31·2 | Inside image |
| − ·00075 | 20·8 | |
| − ·00125 | 13·9 | |
| − ·00175 | 9·7 | |
| − ·00225 | 6·3 | |
| − ·00275 | 3·9 | |
| − ·00325 | 2·6 | |
| − ·00375 | 2·2 | Centre of image |

Similar figures are given by the first method mentioned above.

If the light illuminating the cones be considered it is found that the intensities are as follows:

TABLE 13. Percentage intensity of light falling on retinal cone elements in case of interval between two extended sources. (Pupil diameter, 2 mm.)

| Width | Central cone | 1st row | 2nd row | 3rd row | 4th row |
|---|---|---|---|---|---|
| ·0025 mm. | 33·6 | 85·6 | 97·9 | 99·7 | 100 |
| ·0075 „ | 4·7 | 17 | 83·2 | 97·6 | 99·7 |

The figures show the following points of interest. Firstly, when the geometrical image of the interval on the retina is equal in width to the diameter of a cone the intensity of light falling on the central row of cones is considerable; calculation shows it to be more than one-third the intensity of the images of the sources themselves. As the interval becomes smaller the intensity on the central cone rapidly increases. A limit is therefore very quickly reached beyond which it is impossible for the presence of the interval to be recognised. It will be seen, therefore, that there is a fundamental difference between the case of a small interval between two extended sources and that of a small source on a black ground. In the latter case as the magnitude of the source diminishes an increase in brightness will produce precisely the same range of intensities on the retina; it is thus that the existence of minute points of light is capable of recognition. In the case of the interval (black point or line on bright ground) no such compensation can occur by increasing the blackness of the image or the intensity of illumination of the background. If the dimensions be less than a certain amount then resolution cannot occur. (I find that this difference between the cases of black and white objects has been previously pointed out by Parsons (19). This evidence therefore confirms his conclusion.)

## 5. EFFECTS OF COLOUR MIXTURE IN THE IMAGES PRODUCED BY A LENS UNCORRECTED FOR CHROMATIC DIFFERENCE OF FOCUS.

### *Point source.*

It has been shown in the previous sections that the diameter of an aberration disc is determined by the size of the pupil and the wave length of the light from which it is formed; also that the images of external objects are composed of a series of such discs, differing in size, intensity and colour superimposed on one another. Since the colour and intensity of the discs are known, it is possible to determine approximately by calculation the results of colour mixture in different parts of the image. And further, it will be shown in this section that the conclusions reached may be checked by actual observation. The figures in Table 14 show the wave lengths and intensities of the rays forming discs of given size; also, the mean wave length and amount of white light present as the result of the mixture of two rays which form discs of the same diameter.

TABLE 14. Luminosity of white, mean wave length and colour produced by colour mixture in aberration discs.

| Diameter of disc in mm. | Total luminosity | White luminosity | Mean wave length | Colour |
|---|---|---|---|---|
| 0 | 196 | 0 | 5800 | yellow |
| ·001 | 190 | 0 | 5800 | ,, |
| ·002 | 176 | 0 | 5810 | ,, |
| ·003 | 155 | 0 | 5830 | ,, |
| ·004 | 124 | 0 | 5835 | ,, |
| ·005 | 89 | 6 | 5930 | ,, |
| ·006 | 54 | 13 | 5600 | yellow-greenish white |
| ·007 | 28 | 11 | 5480 | greenish white |
| ·008 | 15 | 10 | 5080 | blue-greenish white |
| ·009 | 9 | 4 | 4920 | bluish white |
| ·010 | 5 | 0 | — | purple |
| ·011 | 3·2 | 0 | — | ,, |
| ·012 | 2·3 | 0 | 4740 | blue |
| ·014 | 1·7 | 0 | 4620 | ,, |
| ·028 | 0 | 0 | 3970 | violet |

The figures were calculated from colour mixture diagrams, based on Newton's centre of mass hypothesis (20). For further details see Table 3.

In making this calculation it was assumed that the centres of the different aberration discs exactly coincided on the retina. Owing to the optic axis and the line of fixation not coinciding this is not actually the case (21). The following experiment shows, however, that the discrepancy is small. In front of a brilliant point source is placed a colour filter transmitting rays corresponding to the ends of the spectrum, green, yellow and orange rays being absorbed. A piece of deep cobalt blue glass is admirable for the purpose. If the source is now examined by the emmetropic eye at a distance, a sharply focussed red point is seen which is surrounded by a blue-violet aberration disc. If the decentering of the discs was of a noticeable amount it would show itself by the want of coincidence between the centre of the violet aberration disc and the red image.

On performing the experiment I found the violet aberration disc to be broken up into a series of radiating bands the ends of which seemed to be of equal length in relationship to the sharply focussed red centre of the image. As far as could be judged therefore no eccentricity is to be observed. The questions relating to the position of the optical centre of the retina in reference to the centre of the fovea will be found mentioned again in § 7.

Now the figures in the above table show that discs of smaller diameter than ·006 mm. are formed largely of rays that, as the result of colour mixture, appear yellow to the eye. Discs of approximately ·008 mm. are formed of rays that are physiologically complementary to one another; a considerable amount (nearly 40 °/₀) of white light is therefore produced. The predominance of the green component causes the white to have a greenish tint; which is not so marked as the intensities would seem to show because in red and green complementary colours the green component is always the brighter.

Discs larger than ·008 mm. in diameter are of a blue or violet colour and as previously shown their intensity is feeble.

I have pointed out in the previous section that the aberration image is built up by the superposition of the series of aberration discs which are produced by rays corresponding to the different regions of the spectrum. The colours at different points of the image can therefore be obtained by a process of summation in a similar manner to intensity. The values obtained are shown in the table below.

TABLE 15 A. Mean wave length, colour, and intensity of light at different points in the aberration image of a point source.

| Distance from centre of image | Total luminosity | White luminosity | Mean wave length | Colour |
|---|---|---|---|---|
| 0 | ∞ | 0 | 5800 | yellow |
| ·036 | 340 | 0 | 5800 | ” |
| ·072 | 130 | ·4 | 5800 | ” |
| ·107 | 53 | 1·5 | 5800 | ” |
| ·143 | 23 | 2·5 | 5800 | ” |
| ·178 | 10 | 2·0 | 5750 | yellowish white |
| ·214 | 3·8 | 1·3 | 5000 | greenish white |
| ·250 | 1·8 | ·8 | 4900 | ” |
| ·286 | ·8 | ·4 | 4850 | blue-greenish white |
| ·321 | ·4 | ·1 | 4800 | bluish white |
| ·36 | ·2 | 0 | 4800 | purple |
| ·39 | ·13 | 0 | 4800 | ” |
| ·43 | ·06 | 0 | 4700 | blue |
| ·50 | — | 0 | 4600 | ” |
| 1·0 | — | 0 | 4000 | violet |

It will be seen that the above calculation gives results of considerable interest. The centre of the image has approximately the same mean wave length as that of the most sharply focussed colour; this has been shown in § 2 to be yellow of 5800 A.U. approximate wave length. From the centre to approximately one-fifth the maximum diameter of the image the colour remains yellow of nearly the same wave length but there is a gradual increase in the amount of the white light present in the image. At ·28 the image is bluish white, the white component having reached its maximum value in comparison with the total intensity present. The amount of the white valency apparently present to the eye is probably greater than the figures would suggest because it is found by most observers that the intensity of the white light produced by colour mixture is greater than that of the sum of the intensities of the two complimentary components (23).

From ·28 to ·36 of the maximum diameter of the image the white intensity falls rapidly to insignificance; the colour of the image being blue-violet. We may therefore regard white light as being produced in two ways in the image of a point source.

(*a*) By the coincidence of aberration discs of the same diameter, *e.g.* red and blue-green.

(*b*) By the overlap of aberration discs of different diameter, but the same centre, *e.g.* yellow discs of small diameter on blue and violet discs of considerable diameter.

If the colour of the light stimulating the different cones be considered in a similar way to intensity in the previous section the following results are obtained.

TABLE 15 B.

| Diameter of pupil | Central cone | | 1st ring | | 2nd ring | | 3rd ring | |
|---|---|---|---|---|---|---|---|---|
| | Intensity | Colour | Intensity | Colour | Intensity | Colour | Intensity | Colour |
| ·9 mm. | 76·4 % | yellow | 3 % | yellowish-white | ·18 % | yellowish-white | — % | bluish-white |
| 2 „ | 46 | „ | 5·5 | yellow | ·9 | yellow | ·07 | yellowish-white |
| 3 „ | 32 | „ | 5·1 | „ | 1·5 | „ | ·45 | yellow |
| 4 „ | 30 | „ | 5 | „ | 1·8 | „ | ·7 | „ |

The figures in the table show that in the case of a point source yellow rays take a predominant part in forming the stimulus to the cones on which the bright central portion of the image falls. Thus in the case of a pupil of 2 mm. diameter the rays stimulating the 3rd ring of cones are still principally yellow in colour, while the intensity has fallen to ·07 %. The intensity falling on succeeding rows is still lower and it is very doubtful if it can cause stimulation. It would seem necessary under these circumstances for such sources to appear yellow in colour to the eye; but such an effect is certainly not noticeable under ordinary circumstances.

The explanations that can be advanced for the apparent want of confirmation of this deduction will be given towards the end of this section.

### *Linear source.*

The colour distribution in the case of the linear source can be deduced approximately from that found for a point source; since a linear source can be regarded as being formed of an infinite series of point sources placed in contiguity. Now it will be at once seen that an entirely new series of conditions are introduced. Whereas in the case of the point source all the centres of the aberration discs coincide approximately—the want of coincidence being negligible in amount—with a linear source there is overlap of aberration discs the centres of which do not coincide. Taking the following case as example, the yellow area at the centre of the image of one part of the source is overlapped by the blue-violet aberration discs formed by rays emanating from parts of the source on either side. In this way a large additional amount of white light is produced. White light is therefore formed in three ways in the image of a linear source: (*a*) and (*b*) those given in the case of a point source above; (*c*) by the overlap of aberration discs of different diameter and different centres; the line joining the centres being parallel to the long axis of the light source and its image.

The amount of white light present in the aberration image of a linear source is therefore considerably greater than that of a point source. The less beneficial form of the intensity curve in the case of the linear source, as shown in the previous section, is probably to a large extent compensated by this increase in the amount of white light present in the image.

The distribution of colour in the image in the plane at right angles to the long axis of the source is otherwise very similar to that found in the case of the point source, that is to say the area near the centre of the image is yellow, while the outer portions are blue and violet.

### *Light source of appreciable size.*

As pointed out in § 3 the source of appreciable size may be regarded as being composed of an infinite number of linear sources placed in juxtaposition so as to cover a small limited area. It will be seen at once that a fourth method (*d*) is introduced by means of which white light is formed, namely by the superposition of aberration discs of different diameter and different centre; the centres lying on lines that are not parallel with the long axis of the light source or its image. An additional amount of white light is thus introduced in the image which may under certain circumstances reach a maximum with the total exclusion of colour. These conditions will now be considered.

Since white light from a single point is refracted by the eye to form an aberration image which varies in colour and intensity from part to part; to reform white light unmixed with colour it is necessary to take light from every part of the aberration image, so that on a single point of the retina there falls not only rays corresponding to the central yellow area of the aberration image, but also those corresponding to every other part of it, including the blue and violet rays which form the largest diameter aberration discs. Now these will be formed by rays which come from points whose images correspond to an area equal in diameter to the violet discs themselves. The white centre therefore appears in an image when its diameter is equal to or greater than that of the largest aberration disc and any further increase in the size of the object is accompanied by a corresponding increase in the central white area. The importance of this deduction will be at once appreciated as it explains how it is that an extended source of white light remains white unaffected by chromatic aberration.

### *Extended light source.*

Since the extended light source produces an image in the retina that is large compared to the diameter of the largest aberration disc the greater part of its surface will be unaffected in colour by chromatic aberration as I have just explained. While at the edge of the image are found fringes which are due to the absence of succeeding aberration images which would obliterate them by the formation of white light.

It was shown in § 4 that the intensity at the edge of the image of an extended light source suffers a sudden diminution at that point which corresponds to the edge of the geometrical image and further that the intensity curve is symmetrical about this point. Further the total width of the fringe is equal to the maximum diameter of the aberration image of a point source. It was also shown when considering cases *A* and *B* of this section (5) that at the centre of the aberration image there is a considerable area which is yellow in colour. Since this is placed symmetrically about the centre of the image one half of this area lies to one side of the mid-point. Now considering the case of the rays proceeding from the extreme edge of an extended light source these will form aberration images whose centre coincides with the geometrical edge of the image. There will be therefore found one-half of these yellow aberration images on either side of the point of maximum intensity change; the inference from this being that yellow extends into the violet fringe a distance equal to one-tenth the maximum width of the fringe. This is not confirmed by experiment, for it is not possible to detect any yellow colouration in the outer half of the fringe. Suppose for the sake of argument, however, that the inner part of the fringe is yellow and that sources of the same intensity are brought into contact to form one uniform illuminated area, then at the line of junction the yellow areas produced by the edge rays should overlap to form a yellow band. This obviously is not the case and therefore the supposition is false, and the yellow colouration therefore cannot extend into the outside violet fringe. The absence of this yellow colouration has therefore to be explained.

If we observe the way in which the rays from successive points near the edge of an illuminated area form their aberration images it will be noticed that the yellow areas at the centre of those images produced by the edge rays are overlapped by the blue and violet portions of all the succeeding images as one proceeds inwards for a distance corresponding to one half their maximum image diameter. Further the intensities of the yellow and blue-violet components must be such that together they produce white light. Now the intensity of the yellow area is not uniform for it rises abruptly to infinity at its centre to fall sharply away again. The intensity of the blue-violet area on the other hand is almost uniform. It follows therefore that the portion of the area corresponding to the central peak will be yellow, surrounding this will be a narrow neutral area, which is formed when the intensities of the components are such that they are complementary to one another; while the rest of the area will be blue-violet mixed with white. Since the sharp central peak is produced by the rays at the edge of the source this lies just within its geometrical image; it therefore follows that no portion of the yellow colouration should be found to the outside of the geometrical edge that is within the region of the violet fringe. The absence of the yellow colouration found by experiment is therefore explained.

Experiment shows that there is another way of regarding the building up of the colouration in the aberration image. An ordinary telescope is taken fitted with an uncolour-corrected object glass. In front of the object glass is placed a metal disc near the edge of which two vertical slits are placed. The disc is now adjusted till the apertures allow light to strike at positions near either edge of the object glass.

If now a linear source be observed through the eyepiece (the long axis of the source being parallel to the slits) it will be found that two narrow spectra occupy the field of view. These spectra are reversed in direction to one another and are found to move over one another, as the focus of the telescope is changed. If the telescope is previously focussed for white light (or for yellow rays by means of a sodium flame or suitable colour filter) then it will be found that the portions of the spectra which overlap have combined to form a single yellow band of considerable brilliance. On either side of this being two purple and violet fringes formed by the ends of the spectra. The yellow band is formed by the mixture of rays corresponding to the green, yellow, orange and part of the red regions of the spectrum (24). A considerable amount of white light is also re-formed by the overlap of the spectra (25). If the distance between the slits placed before the objective are altered the dispersions of the spectra are found to vary in the same manner, but the yellow regions of the spectrum continue to correspond unless the lens suffers from a high degree of spherical aberration. It will be seen that this experiment illustrates another way in which the yellow area at the

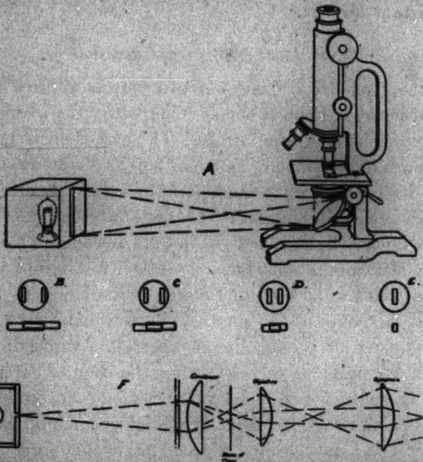


Fig. 9. *A*, arrangement of light source and microscope with Abbe condenser. *B*, *C*, *D*, and *E*, position of slots in diaphragm and appearance of spectra. *F* shows diagrammatically the direction taken by light rays through the lens system of the microscope.

centre of an aberration image and the blue-violet fringe surrounding it are formed. The experiment can be varied in a number of ways. A microscope with an ordinary Abbe condenser can be used instead of the telescope, or indeed the experiment may be performed by the unaided eye, if a metal plate with fine parallel slits is held in front of it. Considerable patience is required if the last method is to succeed, because of the small scale of the spectra. The insertion of a piece of pale cobalt blue glass between the source and the eye greatly increases the facility of observation.

Fig. 9 shows the method of setting up the apparatus in the case of the microscope and the appearances of the spectra with the slits at different distances apart.

### *Interval between two extended sources.*

When the geometrical images of two sources approach one another the interval between them becomes coloured by the overlap of the two external blue-violet aberration fringes. These two fringes meet, when the distance apart of the images is equal to the diameter of the largest aberration disc, so that when the separation is less than this the interval has everywhere light falling on it of a certain intensity. (The intensity values have already been considered in § 4.) Because of this the interval has a blue-violet colouration which increases in luminosity as the interval decreases in width or as the pupil increases in diameter. Externally to the interval the edges of the sources will each have a yellow fringe of half the diameter of the maximum aberration discs, in the manner described in the previous sub-section for the edge of extended sources. The colour in the image of an interval between two sources can also be regarded as being the compliment of a bright source of the same width as the interval on a dark ground, since together they form one uniformly illuminated surface. The yellow colouration of the centre of the image of the bright source and the blue-violet fringe which bound it on either side have their counterpart in the blue-violet centre to the image of an interval as the yellow surrounding fringes. Not only are the colours complementary to one another, but also the intensities at different points of the images as shown in the previous section (4).

Identically the same questions therefore arise in respect to the colour of small intervals as was pointed out above in the case of point and linear source; namely, how is it that these colours are not observed by the eye under ordinary everyday conditions of vision? This question will now receive consideration.

*Experiments to determine how it comes about that small sources and small intervals between sources do not appear coloured in ordinary vision.* At the commencement, confirmation was sought experimentally for the presence of the colours and fringes deduced from the hypothesis. This was done as follows:

An ordinary terrestrial telescope was taken, the achromatic object glass of which had been replaced by a single crown glass lens, of approximately the same focal length. Through it were examined a number of fine white lines drawn on a black background. When the lines were in focus they were found to be strongly coloured, the actual tint varying as the focus was changed. Surrounding them were noticed the faintly coloured fringes which spread for a considerable distance from the lines over the black surface. Calculation showed that the coloured images thus presented to the eye had a magnitude 20 times those normally formed with a pupil of 1 mm. It was possible therefore to examine closely the way chromatic images are built up.

The first experiments to be performed with the telescope were made to determine the effect of change of focus on the colour and apparent sharpness of the images. It was found that when the image was sharpest the lines appeared yellow and the fringes deep blue. When inside this focus the lines appeared apple-green and the fringes purple. Within these limits the sharpness of the lines did not appear to suffer very noticeably, but if the focus was sufficiently changed so that the lines appeared green or red then the fringes became very prominent and the edges of the lines appeared blurred. This experiment showed that the yellow area at the centre of the image does exist, and also that the colour is of considerable strength. An explanation must therefore be sought as to why this colouration of fine white objects does not obtrude itself in ordinary vision. The following hypotheses can be tentatively advanced:

(1) That the size of the fringe is so small in the case of the image of a point source that the whole of it falls on a single cone. The colours of the fringe, therefore, re-combine physiologically within the cone to give the impression of a white image.

(2) That the yellow colouration of the centre of the fringe is not perceived because under the conditions that exist the eye cannot differentiate between a white and yellow image.

(3) That the violet fringe is perceived by neighbouring cones, the stimulus received by them being combined physiologically to give the sensation of a single white image, in nerve centres situated between the end organs and the higher brain centres, where conscious perception takes place.

Now the size of the fringe has been calculated earlier in this paper. With a pupil of 4 mm. the lowest estimate (that of Helmholtz) is ·0426 mm. The distance between neighbouring cones is ·0025 mm. approximately. The fringe, therefore, stimulates about 17 cones width when the pupil is 4 mm. diameter. This disposes of the first hypothesis. To test the second and third hypotheses the following experiments were performed. About 4 metres from an ordinary incandescent gas mantle was placed a fine vertical polished steel wire. In this was seen a sharp narrow image of the light source. To one side of it was formed an image by direct reflection at the surface of a piece of black glass. When the images were carefully examined there did not appear to be any obvious difference in their colours. The fine image showed, however, slight fluctuations in tint from red to green, a phenomenon probably due to change of focus of the eye. A colour filter absorbing the violet and part of the blue end of the spectrum was now placed before the eye. The large image was found to obviously change in colour whereas the fine image did not change noticeably.

The experiment was repeated using a more powerful light source, also with a larger gauge steel wire and it was now found that a noticeable change occurred when the yellow colour filter was introduced. It would seem, therefore, that when the intensity was low and when the object is of small dimensions the eye cannot differentiate between white and yellow whereas when the intensity is high or the size of the object considerable, differentiation is possible.

A further experiment was performed that confirmed this view. A strand of white sewing cotton about ·4 mm. in diameter was painted for a portion of its length a strong lemon colour with barium chromate pigment. The strand was then placed in daylight in front of a black background and the distance from the eye gradually increased. When close to the eye the coloured portions were clearly differentiated. At a distance of two metres colour differences were noticeably less distinct. At ten metres it was not possible to identify them. Similar tests with red and green showed that at ten metres they could still be recognised. This experiment was now repeated in weak daylight and it was found that the coloured portions of the cotton became indistinguishable from the rest at a distance of three metres. In sunlight on the other hand the colour was recognisable at 20 metres. The intensity clearly plays an important part in deciding the distance at which the colour could be identified. This might be due to change in size of the pupil since the pupil would increase in diameter as the illumination decreased. This increase in diameter of pupil would increase the diameter of the fringe and would therefore change the distribution of colour in the image. The effect of diameter of pupil was investigated by placing a series of apertures in front of the eye, the distance being measured at which the coloured portions of the cotton strand could be recognised. It was found that limiting the diameter of the pupil far from increasing the distance at which the colour could be distinguished actually had the effect of decreasing it, which shows conclusively that it is intensity that controls directly the clearness of perception of small yellow objects.

These experiments show that the second hypothesis suggested above cannot be true. It would appear necessary, therefore, to assume the existence of some process of physiological summation such as that outlined in the third hypothesis above. This would also make it possible to give the following explanation of the effect of intensity on the differentiation of small white and yellow objects. When the intensity is low the violet fringe surrounding the image of a white object is below the threshold for the cones on which it falls, and the absence of the stimulus, therefore, causes white and yellow objects to be seen alike. When it is high, however, the fringe is observable by the eye; white and yellow objects, therefore, look different in colour. This view is borne out by the proportion that exists between the intensity of illumination and the distance at which small white and yellow objects can be recognised.

In § 4 I have assumed that the peripheral parts of an aberration image do not tend to spoil the definition, because under normal circumstances they do not reach consciousness. In this section we have seen, however, that one is forced to conclude that the peripheral parts of the image are perceived, for otherwise we cannot explain how the eye can differentiate between small white and yellow sources. These two conclusions would appear to be mutually antagonistic. Their simultaneous existence may be explained, however, as follows:

Experiments on binocular antagonism have shown that although one image may be suppressed by another of greater interest, yet immediately any alteration is made in the suppressed image the fact is immediately observed. The suppressed image, therefore, stimulates the organ of vision and its form is appreciated although it does not reach consciousness.

Further, it can be shown by experiment that if two images of the same degree of interest be presented to the eye, the one sharply in focus and the other out of focus, that the sharp image suppresses the other. Therefore applying these data to the problem of the images produced by a lens suffering from chromatic aberration, suppose the image to be arbitrarily divided into two parts, a sharp yellow image and a diffuse blue one, the yellow image being sharper and brighter should cause the suppression of the blue-violet one and as a result the image should appear sharp. Although the blue-violet fringe image does not reach consciousness, it is still appreciated by the organ of vision, and therefore its presence or absence is at once notified to the higher centres. When it is present the object is interpreted as being "white," whereas when it is absent the object is seen as yellow. If the blue image is not perceived because its intensity is so low that it fails to act as an adequate stimulus, then as experiment shows small yellow and white sources appear alike.

Where the size of the object is considerable, superposition of the violet edges and the central yellow bands of the fringes will take place with the re-formation of white light. The apparent colour of the white object should, therefore, diminish as its width increases. Experiment with the chromatic telescope shows that when the width of the image is equal to that of the fringe the yellow area at the centre of the fringe commences to divide into two separate half fringes leaving a pure white area at the centre. This now increases in width as the object increases. The two yellow half fringes thus formed on the inside of the edge of the image are the same width as the blue-violet ones on the outside. The latter do not vary appreciably in width with the size of the white object. The colours of both fringes vary with the focus of the telescope. When at the position of best focus the internal half fringe is pale lemon-yellow, the external half being blue-violet with a narrow purple margin close to the true edge of the image. Outside this focus the internal half fringe becomes reddish, the external bright blue. Inside this focus the internal half fringe becomes apple-green, the external purple. These modifications of the appearance of the fringe are in agreement with the explanation of the fringe formation described above.

The presence of these coloured fringes round the edges of white objects are not observed by the eye, for the reasons that have already been given.

In the case of a small interval between two sources experiment shows that the intensity of illumination plays a part as important in the differentiation of violet and black as it did in the case of white and yellow. The suggestions that can be made for the non-observance with the unaided eye of the blue colouration of small black images are also very similar. As in the case of white and yellow some subconscious recognition of the presence or absence of the yellow fringe must cause differentiation between black and violet. This is only found to occur when the light intensity is sufficient to cause the fringe to act as an adequate stimulus to the retina.

### 6. EFFECT OF CERTAIN PHYSICAL AND PHYSIOLOGICAL FACTORS ON THE APPEARANCE OF FRINGES PRODUCED BY CHROMATIC ABERRATION.

Helmholtz pointed out when describing his hypothesis that there are two factors which reduce to a considerable extent the magnitude of the effects of chromatic aberration, as they appear to the eye.

(1) The greater luminosity of the rays corresponding to the middle region of the spectrum.

(2) The far larger area that has to be illuminated by rays which correspond to the ends of the spectrum.

The intensity curves in Fig. 10 illustrate clearly the importance of these two factors.

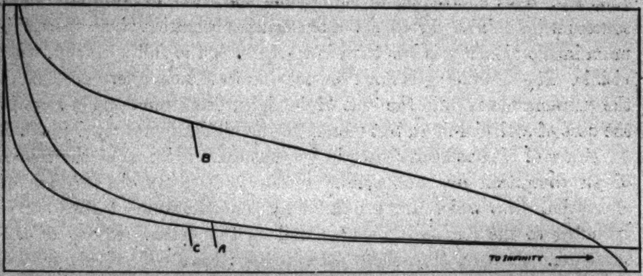


Fig. 10. Curve (*A*) shows the intensity distribution in the case of a point source from which the effects of variation of luminosity are excluded. (Factor (1) above.) Curve (*B*) shows the intensity distribution in the case of a point source from which the effects of the size of aberration discs are excluded. (Factor (2) above.) Curve (*C*) shows the normal intensity curve in the case of the point source.

It will be observed that while both factors are of the greatest importance, the variation in size of the aberration discs has the greater effect.

I have found that in addition to those just mentioned there are a number of other factors which operate in such a way as to reduce the visibility of the fringes caused by chromatic aberration. Some of these are physical and some physiological.

(3) The shortened spectrum that ordinary light sources are found to possess.

(4) The order of the colours in the spectrum.

(5) The position in the spectrum of the rays selected to form the most sharply focussed image.

(6) The variation of dispersion with wave length of the eye media.

(7) The shape of the pupil.

(8) The effects of simultaneous contrast.

(9) Antagonism between focussed and unfocussed image.

(10) The effect of threshold of eye for light of low intensity.

(11) Chromatic difference of magnification (§ 8).

These factors will now be briefly considered.

*Factor* 3. The visible spectrum extends from approximately 3800 A.U. to 7800 A.U. If, however, the spectrum of daylight be examined with an ordinary spectroscope, it will be found that the red end is shorter than that given above, as a rule it does not extend much further than 7300 A.U. Similarly artificial light sources give a spectrum which is shortened at the violet end, the extreme limit being approximately 4200 A.U. This shortening of the visible spectrum for ordinary light sources will diminish the effects of chromatic aberration since the maximum image diameter is less than it would be if the full spectrum were visible. The shortening is however not due to the complete absence of the extreme red rays in the case of daylight, or extreme violet rays in the case of artificial light, but merely to the diminished intensity (26).

*Factor* 4. It has been shown in § 4 that there is found at the centre of an aberration image a yellow nucleus formed by the overlap of aberration discs which are produced by the refraction of rays corresponding to the red and green as well as the yellow regions of the spectrum. Further, that these three regions have collectively by far the greater portion of the luminosity of the spectrum; their association together is therefore most fortunate. Not only is the intensity of the image formed by them high in value, and its colour nearest in its approach to white light of all the spectral colours, but also a considerable proportion of white light is formed by the mixture of its constituent rays.

*Factor* 5. In a spectrum of uniform dispersion such as is produced by a diffraction grating the best position for the rays of maximum luminosity would be at the centre of the spectrum (namely at 5650 A.U.), for under these circumstances the eye would focus these rays sharply on the retina, and therefore the aberration discs produced by rays which correspond to the ends of the spectrum would have their least diameter. Owing, however, to the dispersion of glass being less at the red end of the spectrum than at the violet, greater benefit would accrue by moving the maximum to the red side of the centre. Abney showed the point of maximum luminosity to be at 5950 A.U. in the case of the arc lamp. This point is to the red side of the centre and is therefore found to be approximately at the best position in the spectrum.

*Factor* 6. In the spectrum formed by refraction at the surface of the transparent media which form a prism or lens, the red end of the spectrum is relatively less dispersed than the violet. This causes the yellow nucleus formed at the centre of an aberration image to be relatively narrower and the violet fringe surrounding it to be correspondingly broader than they would be if the dispersion throughout the spectrum were uniform. This has the effect of concentrating the light at the centre of the image and reducing it towards the edges, thus decreasing the effects of chromatic aberration.

*Factor* 7. Calculation shows that the shape of the pupil has a considerable effect on the intensity of the peripheral parts of an aberration image. Consider for example the case of a linear source; if the pupil were square, two sides being parallel with the source, then the image would be built up of square aberration discs. Comparing the cases of a square and a circular pupil of the same area, the intensity of the aberration discs formed by a ray of given wave length is the same since the area of the discs are equal. Now circle and square discs correspond in width at a distance from the centre of the image equal to approximately one quarter the side of the square disc. A point situated at this distance from the centre of the image will therefore receive the same illumination whether the pupil be square or circular. The illumination at the centre of the image will, however, be reduced by 14 % approximately, if a circular pupil be replaced by a square, whereas the intensity of the outer portions of the image will be increased under the same circumstances. The circular pupil is therefore more beneficial than the square from the point of view of chromatic aberration.

*Factor* 8. Simultaneous contrast has three principal effects on the images presented to the eye. (*a*) It increases differences of intensity. (*b*) It accentuates differences of colour. (*c*) Experiments on absorption bands have shown that it modifies considerably the apparent slope in an intensity gradient. The changes have been found to be most marked in those cases where there is a discontinuity in the intensity curve (15).

All these effects can be claimed with certainty to have a very important action on the apparent intensity distribution in an aberration image. To take for example the case of a point source of light, it has been shown in § 4 that the image consists of a central point at which the intensity reaches a very high value, there being a rapid fall in the intensity in every direction. Contrast will have the effect of making the central intensity appear greater and that of the periphery less in amount. It will therefore cause the image to seem sharper and more brilliant to the eye. Experiment shows that the magnitude of the changes brought about by contrast increases greatly, as the surfaces suffering contrast approach one another. The improvements in the aberration image caused by the phenomenon are probably therefore very considerable owing to the rapidity with which the intensity changes in value between neighbouring cones of the retina.

With regard to colour it has been shown (in § 5) that at the centre of the aberration image there is formed a yellow area bounded on either side by a blue-violet fringe. The effect of contrast on the colours will be to increase apparently the saturation of the two colours since they are approximately complementary to one another. This effect is not beneficial, however, the way in which it escapes observation under ordinary circumstances has already been considered in the same section.

The case of the intensity curve at the edge of an extended source is of particular interest. It was pointed out by Helmholtz that according to calculation there is a sharp fall in the intensity value at that point of the image which corresponds in position with the edge of the geometrical image of the source. This deduction has been confirmed by the author (see § 4). It has also been shown (in § 5) that the colours of the fringes, produced by chromatic aberration on either side of this boundary line are complementary to one another. Contrast will, therefore, have two effects; it will tend to increase the apparent difference between the intensity values, thus making the image appear sharper and more distinct to the eye, and it will tend to accentuate the saturations of the coloured fringes.

*Factor* 9. In a previous paper a connection has been traced between antagonism and simultaneous contrast (37). In dealing with its effects on chromatic aberration, however, it is more convenient to consider the two factors separately. Thus if two images be presented to the eye, one of which is sharp and the other diffuse, it can be proved by experiment that the sharp image reaches consciousness at the expense of the diffuse one. In the case of an image formed by a lens suffering from chromatic aberration, the case is one where a relatively sharp yellow image and a diffuse violet image are superimposed. The effects of antagonism are such as to cause the sharp yellow image to reach consciousness at the expense of the diffuse violet one. The relatively greater intensity of the yellow image also still further increases its visibility, and causes the suppression of the violet fringe.

*Factor* 10. It is well known that if the intensity of an image falling on the retina has less than a certain value, the image does not act as a stimulus to the retina. This minimal value that will just produce stimulation is known as the threshold of vision. The value of the threshold is increased if the eye be exposed to a bright light. When the eye is light adapted the threshold is less for the red end of the spectrum than it is for the violet. The reverse is, however, the case when dark adaptation of the eye has taken place. Owing to the high intensity of the image falling on the eye when daylight or bright artificial light is used the violet fringe surrounding the image is to a considerable extent below the threshold of the vision. The relatively greater dispersion of the violet end of the spectrum tends to accentuate this effect.

### 7. CHROMATIC ABERRATION WITH NIGHT VISION.

When the intensity of illumination is diminished several changes occur in the conditions of vision which have important effects on chromatic aberration.

Firstly, the pupil increases in diameter, thus increasing at the same time the diameters of all the aberration discs, and therefore also the apparent dimensions of the image.

Secondly, a change occurs in the apparent luminosity of the spectrum, which causes it to appear shorter; the change being due to the rays at the ends of the spectrum ceasing to be visible. These changes affect particularly the rays of long wave length. The point of greatest apparent luminosity is found also to move towards the green (*i.e.* corresponds to rays of shorter wave length). These changes are known collectively as the phenomena of Purkinji. If the intensity is reduced still further the whole spectrum bcomes monochromatic, the point of maximum luminosity being found at about 5300 A.U. As the intensity of illumination is decreased, therefore, the rays sharply focussed on the retina will, according to the hypotheses advanced in § 2, correspond to rays of shorter wave length than those found for normal illumination. At the same time the aberration discs of larger diameter will disappear because the rays forming them no longer act as adequate stimuli to the retina; this statement applying particularly to rays from the red end of the spectrum.

In order to show more clearly these effects on chromatic aberration the intensities of aberration discs of different diameter will now be considered in the case of a point source, exactly the same methods being applied as in § 4, above.

Table 16. Intensity and total luminosity of aberration discs of different diameter, with night vision.

(The pupil diameter is assumed to be 1 mm.)

| Diameter of image in mm. | Total luminosity | Intensity |
|---|---|---|
| 0 | 196 | ∞ |
| ·001 | 186 | 230 |
| ·002 | 158 | 51·4 |
| ·003 | 121 | 16·9 |
| ·004 | 93 | 7·6 |
| ·005 | 61 | 3·2 |
| ·006 | 53 | 1·9 |
| ·007 | 38 | 1·0 |
| ·008 | 24 | ·7 |
| ·009 | 15 | ·3 |
| ·010 | 11 | ·13 |
| ·012 | 5 | ·06 |
| ·014 | 1·3 | ·015 |
| ·028 | 0 | 0 |

If Table 16 be compared with that given for day vision (Table 3) it will be seen that the values are equal for aberration disc of approximately ·006 mm. in diameter. Between this and ·001 mm. the discs are less intense with night vision, whereas above ·006 mm. the reverse is the case. It is to be expected, however, that the larger discs owing to their low intensity will be beneath the visual threshold, in the same way as they are in day vision but to a greater degree. The greater intensity of the more peripheral discs is not, therefore, so great a disadvantage as it would appear. The less intensity of the discs between ·001 and ·006 mm. diameter is beneficial since it makes the image appear sharper owing to the relatively greater intensity of the central discs. In this calculation the luminosity values of the scotopic eye given by Schaternikoff are used. Those of v. Kries give a similar result. Haycraft's(28) curves would indicate even more favourable conditions with night vision. These circumstances would point to the effects of chromatic aberration being about 20 to 30 % less with night vision than with day.

A still further increase in sharpness of the image is caused by the change in the wave length of the light with the greatest apparent luminosity. For night vision this is approximately 12 % shorter in wave length than that found for day. This reduces the effects of diffraction by a similar amount and therefore materially increases the sharpness of the image. Two other factors of importance remain to be considered. Since the foveal area of the retina which contains cone elements only is almost completely blind in night vision, the retinal areas peripheral to the fovea are alone available, with feeble illumination. The images utilised are therefore formed by rays of considerable obliquity with the optical axis of the eye. Spherical aberration and astigmatism are therefore introduced. The effects of chromatic difference of magnification have also to be considered; this factor will be more fully dealt with in the next section.

These effects must cause a decrease in the definition of the aberration image, which is still further added to by the large pupil diameters that are employed in a dim light. At the same time, however, the use of the rod retinal apparatus must reduce to a great extent the necessity for the formation of sharp pictures, since it has been proved by histological investigation that whereas the cones have each their own second order neurons, with which their dendritic processes anastomose, the rods send processes which ramify freely among many neuron cells. The differentiation of the nerve paths would, therefore, appear to be of a much lower order in the case of the rods. From this it would follow that even under ideal conditions for the formation of sharp images, the appreciation of form would be less exact with the rod apparatus than with that of the cones. This view receives support from the results of experiment, for even when aberrations are reduced by the use of a small artificial pupil (1 mm.) the visual acuity of the retina peripheral to the fovea is still relatively of a very low order under conditions of reduced illumination.

Too much reliance should not be placed on the above parallelism, for it is equally possible to argue that if the images formed on the periphery of the retina had been more sharply defined, then the evolution of the rod apparatus would have reached a higher stage of development.

## 8. CHROMATIC DIFFERENCE OF MAGNIFICATION AND CHROMATIC STEREOSCOPY.

Gullstrand(29) has called attention to the fact that optical theory requires the presence of chromatic difference of magnification in the images formed on the retina. According to his estimate the image formed by violet rays is 3 % smaller than that produced by red rays. If an object consisting of three parallel linear light sources forms an image on the retina, and if it be so situated in relationship with the eye that while the central source forms an image that coincides with the optical centre of the retina, the images of the other two sources are formed at a point on the retina one half millimetre distance from the centre, then these will be found to consist of impure spectra approximately ·015 mm. long. Now this length is comparable in magnitude to the diameters of the larger aberration discs (see Table 2) and is roughly equal to six times the diameter of the foveal cones; it would be anticipated, therefore, that the effects of chromatic difference of focus would be easily recognised by the eye. Such is not found to be the case. The two explanations advanced by Gullstrand for their absence may be stated briefly as follows: (1) The magnitude of the effect at the optical centre of the retina is zero. It increases, however, in the same ratio as the distance between the illuminated point and the optical centre of the retina increases. The fovea which is the part of the retina with the greatest visual acuity corresponds approximately in position with the optical centre of the eye. As one proceeds from the fovea towards the periphery of the retina the acuity rapidly decreases. Therefore as the effects of chromatic difference of magnification increase so also does the inability of the eye to distinguish them. (2) The second reason Gullstrand advanced is that refraction of the light rays occurs as they leave the vitreous body and enter the anterior layers of the retina, which bends the rays towards the axis, and is greater for violet rays than for the red rays. This refraction, therefore, tends to diminish the magnitude of chromatic difference of magnification.

Of Gullstrand's explanations the first appeared to me the more plausible; for against the second could be advanced the following objections. Bending towards the axis can only occur if the light rays enter a denser medium on leaving the vitreous body. Such bending cannot be sufficient to render red and violet rays even parallel to one another unless the refractive index of the medium they enter is far higher than that of the nucleus of the lens. Further even if such bending towards the axis such as Gullstrand requires did occur the distance the rays have to travel between the point at which they enter the retinal medium to that at which they strike the sensitive layer is so small (less than ·05 mm.) that the correcting effect would be negligible.

These considerations seem to me to render Gullstrand's second hypothesis untenable. It will be shown at the end of this section that chromatic stereoscopy (30) is probably caused by chromatic difference of magnification. The eye does, under certain conditions at all events, appreciate the relative displacements of the aberration discs produced by rays of different wave length. Therefore **Gullstrand's** first hypothesis would appear not to hold for foveal vision and therefore cannot explain how it is that the definition of images falling on the fovea and the acuity of vision remains unaffected. The explanation of the simultaneous existence of these apparently antagonistic properties will now be given.

### *Distribution of intensity in images produced away from the optical centre of the eye.*

If an image formed by the refracting system of the eye falls on a point one-half millimetre distant from the optical centre of the retina and if yellow rays are regarded as forming the nucleus of this image then the centres of the images formed by other rays of the spectrum will be found at different distances from the nucleus. These distances are shown in the following table (17), alongside them being placed the semi-diameters of the aberration discs for a pupil of 1 mm. diameter.

TABLE 17. Showing distances between nucleus and centres of aberration discs and the semi-diameters of the discs in case of an image formed one-half millimetre from optical centre of retina and with a pupil of one millimetre.

| Wave length in A.U. | Distance between nucleus and centre of disc | Semi-diameter of aberration disc |
|---|---|---|
| 4000 | ·01430 | ·01380 |
| 4500 | ·00810 | ·00790 |
| 5000 | ·00430 | ·00410 |
| 5500 | ·00125 | ·00122 |
| 6000 | ·00102 | ·00102 |
| 6500 | ·00290 | ·00289 |
| 7000 | ·00445 | ·00462 |
| 7500 | ·00590 | ·00605 |

From the figures in this table it will be seen that the semi-diameters of the aberration discs are approximately equal to the distances of the centres of the discs from the yellow nucleus of the image. The latter is therefore just overlapped by the edges of each of the aberration discs. The intensity and colour of the nucleus is therefore the same as it would be if the image had been formed (as described in § 4) at the optical centre of the retina, since the same rays contribute to its formation in both cases.

It is, however, formed in the limiting position for if the image falls on a point at a greater distance than one-half millimetre from the centre of the retina or if the pupil be smaller in size than 1 mm., then overlap does not occur and, as will be shown later, the intensity of the centre of the nucleus is seriously affected. These conditions are found to be very different from those which Gullstrand supposed to exist. He stated that there would be found a gradual degradation of definition on passing outwards from the optical centre of the retina. There is found instead an actual improvement to definition up to a certain point at which the sharpness of the image suddenly breaks down. The form of the intensity curve, therefore, varies with the size of the pupil and the distance from the optical centre of the retina at which the image is formed. It will be seen at once that the aberration discs just coincide with the central nucleus of the image when the pupil diameter is twice the distance of the image from the centre of the retina. Under these conditions it is found that the image takes the form of the figure eight, the yellow nucleus being situated at the point of contact of the circles. The outer circle which is the smaller being formed by red rays, the inner and larger by violet. The intensity and width of image, therefore, vary according to the direction through the nucleus which is considered. The following values may be given as examples:

TABLE 18. Diameter of image and amount of chromatic aberration in case of a point source situated at a distance from the optical centre of the retina equal to one-third the pupil diameter. (The figures in this table are calculated on the basis of the diameter and amount of chromatic aberration of an image situated at the optical centre of the retina being both equal to 100.)

| Direction | Diameter of image | Amount of chromatic aberration |
|---|---|---|
| Radially ... ... ... | 100 % | 96 % |
| Tangentially ... ... | 160 „ | 74 „ |
| 45° ... ... ... | 132 „ | 83 „ |
| Average ... ... ... | 131 „ | 84 „ |

The values show a very remarkable feature in the distribution of intensity, namely, that although the average diameter of the image is larger, and therefore the effects of chromatic aberration greater by a corresponding amount, yet when the intensity distribution near the centre of the image is considered it is found that the definition of the image is actually 15 % better than that of an image formed at the optical centre of the eye. When the fact is recalled that the fovea does not correspond with the optical centre of the retina (being to the temporal side in the emmetropic eye) the significance of the above features is at once appreciated. Estimates of the amount of decentering vary, but if ·6 millimetre be assumed to be the average value then a reduction of 15 % in the chromatic aberration will be found at the centre of the fovea with a pupil of 1·8 mm. It would seem therefore that the presence of chromatic difference of magnification far from being a disadvantage actually causes improvement in visual acuity. As the ratio of diameter of pupil to the distance of the image from the optical centre decreases, the efficiency increases up to a point where the ratio value becomes 2. The efficiency drops at this point to a low value. Where overlap of the aberration discs ceases, therefore, the definition of the image breaks down suddenly and the brightness of the image also suffers. These changes in the definition are illustrated by the figures in Table 19.

TABLE 19. Amount of chromatic aberration and average diameter of the image of a point source at different distances from the optical centre. Chromatic aberration and diameter at optical centre taken as unity.

| Ratio of diameter of pupil to distance of image from optical centre of retina | Average maximum diameter of image | Average amount of chromatic aberration | Efficiency |
|---|---|---|---|
| ∞ | 100 % | 100 % | 1 |
| 4 | 127 | 93 | 1·1 |
| 3 | 133 | 85 | 1·2 |
| 2 | 146 | 100 | 1 |
| 1½ | 200 | 180 | ·55 |
| 1 | 300 | 200 | ·5 |

The effects of chromatic aberration on definition therefore vary with the ratio between the pupil diameter and the distance between the centre of the image and the optical centre of the retina. There is not, however, the uniform increase in the amount of chromatic aberration in the image as the distance from the optical centre of the retina is increased as Gullstrand supposed. But as pointed out above a fall, followed by an abrupt rise, at the point where overlap ceases. This occurs at a considerable distance from the optical centre when the pupil is large and its effects would be serious, but much closer when the pupil is small and its effects therefore almost negligible.

When the source is linear the changes differ according to the axis considered. Thus when the image is radial (horizontal in the case of observer with the fovea to the temporal side of the optical centre of the eye) its definition remains uninfluenced by chromatic difference of magnification. When the image is tangential (vertical in the case just mentioned) the effects should be more clearly marked.

If the fovea is not displaced horizontally to the temporal side of the retinal centre but in some other direction, the axes in which the definition is good and bad will correspondingly alter. It is possible to explain in this way why chromatic stereoscopy which will be shown to follow as an axiom from chromatic difference of focus differs in its degree with different axes and different observers.

### *Chromatic stereoscopy.*

When coloured sources lying on a dark ground are examined binocularly it is found by most observers that they appear to lie in different planes. The further apart in the spectrum the colours are, the greater the amount of relief. With red and blue sources Donders(31) found the red to be apparently 20 cms. in front of the blue at a distance of four metres. The effects vary, however, very much, for while most observers find red to be in front of blue, others see no relief at all, or find the positions reversed. The explanation advanced by Donders, namely, that the appreciation of relief is due to the greater accommodative effort required to focus red rays on the retina, can only explain why red sources should appear in front of blue and cannot give any indication of why relief is sometimes absent or even reversed. Further, it can be shown by testing the eyes separately that the degree of effort required for accommodation does not give any useful indication of distance. Donders' hypothesis is therefore inadequate.

Another explanation was advanced by Einthoven(32). Since the visual axis and the optical axis form an angle with one another (the visual axis being to the temporal side at the retina) and since the former takes its origin from the posterior nodal point, it passes obliquely through the nasal side of the pupil. See Fig. 11 (*A*).

It therefore follows that rays coming to a focus either in front of or behind the retina will form aberration images whose centres will not coincide at the retina. The centre of images produced by blue and violet rays will be nearer to the optical axis than those produced by red rays. Since the fovea in emmetropia is to the temporal side of the optical axes of both eyes the blue source will appear to be further from the eye than the red. In proof of this hypothesis he quoted the following experiments.

If the pupil be dilated with atropine and a small aperture in an opaque disc be placed before each eye then, as the apertures are moved towards or away from one another, the relief of red and blue sources is found to vary. When the apertures are moved as far as possible away from one another, thus exaggerating the effect that takes place in normal vision, the blue image is found to be markedly behind the red. When the apertures are placed as close as possible to one another then the reversed effect is witnessed.

Einthoven's hypothesis would appear therefore to be confirmed by experiment. According to this hypothesis observers who find no chromatic stereoscopy or the colours in reverse order (blue in front of red) have pupils which are central with the visual line or are displaced to the nasal side of it. This may be caused by pupilar eccentricity or to the macular being placed on the nasal side of the optical centre of the retina as occurs in myopia. (Parsons(33).)

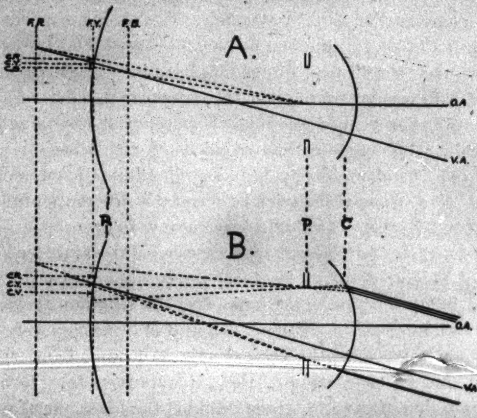


Fig. 11. To show the causes of chromatic difference of magnification (*A*) on Einthoven's basis, (*B*) on the Author's basis. *FR*, *FY* and *FB*, focal planes for red, yellow and blue rays respectively. *CR*, *CV* and *CB*, centres of red, yellow and blue images respectively. *OA*, optical axis. *VA*, visual axis. *R*, retina. *P*, pupil. *C*, cornea.

The following calculation of the amount of chromatic difference of magnification shows, however, that the hypothesis must be rejected. Suppose parallel rays corresponding to the *B* and *H* Fraunhofer lines to be refracted by the eye. If the centre of the fovea be ·6 mm. to the temporal side of the optical centre of the retina and if the distances of the posterior nodal point from the retina and the plane of the iris be 17 and 4 mm. respectively, the apparent decentration of the pupil with regard to the visual line will be ·14 mm. Now it has been shown in § 1 that the focal points of rays corresponding in wave length to the *B* and *H* Fraunhofer lines are ·75 mm. apart (Wolf). The centres of the aberration discs formed by the rays on the retina will, therefore, be approximately ·005 mm. apart; that is, the chromatic difference of magnification is less than 1 % of the distance of the image from the optical centre of the retina. But Gullstrand(29) has estimated this correctly at 3 %, that is at more than three times the value calculated according to Einthoven's hypothesis.

Now my view is that chromatic difference of magnification is not due to the pupil being eccentric to the visual line as thought by Einthoven but to the refracting system of the eye being decentered and tilted in respect to the bundle of rays passing through the pupil to the retina as shown in Fig. 11 (*B*). Assuming Tscherning's constants for the refracting system of the eye to be correct(34) I find by calculation that the chromatic difference of magnification for rays corresponding to the *B* and *H* lines is approximately 3 %, the mean distance of the image from the optical centre of the retina. That confirms the value given by Gullstrand. The value calculated on Einthoven's basis is certainly too low and therefore his explanation of chromatic stereoscopy is erroneous. Now if my explanation of chromatic difference of magnification is accepted then chromatic stereoscopy follows as a necessary deduction, and further, Donders' experimental value (red 20 cms. in front of blue at 4 metres distance) fits in almost exactly with the calculated value for the emmetropic eye. Calculation shows further that the foci for rays of different colour fall approximately along a straight line which cuts the optical axis 11·5 mm. from the retina (assuming Tscherning's values), that is approximately through the centre of rotation of the eye. Rays reaching the fovea therefore passed through the cornea at a point ·5 mm. distant from the optical axis of the eye and to the nasal side. If artificial pupils be placed before the eye and made strongly temporal, the normal relief (red in front of blue) is exaggerated. If placed to the nasal sides then, according to the above hypothesis, the relief should be reversed (blue in front of red). These changes are in accordance with what Einthoven(32) found by experiment to be the case. The hypothesis fits in therefore with the result of experiments. Further it explains why certain observers either see no relief or find it the reverse of normal. Such cases may be due either to eccentricity of the pupil or to the fovea being in the centre or to the nasal side of the retina. In cases where the phenomenon is absent another explanation is possible. Throughout this section it has been assumed that in normal vision the fovea is to the temporal side of the optical centre of the retina. When this is the case the stereoscopic effect is most clearly marked when the sources have vertical contours. If the principal contours are horizontal the effect may be absent even in normal vision.

Another type of chromatic stereoscopy has also been described by Einthoven. This affects monocular vision even, being due as was the type just considered to chromatic difference of magnification and not as Einthoven thought to the angle which the visual and optical axes make with one another. The phenomenon is well seen when vertical red and blue linear patterns are viewed with the light coming from one side. Suppose the left eye be used, and the light be coming from the same side, then the fovea being to the left side of the optical axes, the blue image will be displaced to the right in relationship to the red image. This will cause overlap to occur between this blue image and the red one formed on the retina immediately to the right of it. To the right side of the blue image will, therefore, appear a bright edge. On the left side, however, the displacement of the blue image to the right causes a black interval to appear between the blue image and the red image to the left of it. The same process occurs between all the red and blue images formed on the retina; the latter, therefore, have a bright edge on their right, and a dark edge on their left. These bright and dark edges are seen by the eye, and are referred to the opposite side since the images are reversed on the retina. And since the objects are illuminated from the left the alternate bright and dark edges are judged as being due to relief, and because the bright edge appears on the left of the blue pattern, this appears to lie in front of the red. In the case of the right eye the opposite effect would be seen, the phenomenon is only seen, therefore, with monocular vision.

## 9. EFFECT OF CHROMATIC ABERRATION ON THE IMAGES OF COLOURED OBJECTS.

In the preceding sections, sources emitting white light have alone been considered and it has been shown that not only are strongly coloured fringes formed round the edges of sources by chromatic aberration but also that under certain circumstances there is considerable difficulty in differentiating between small coloured and colourless sources. In the case of coloured sources, therefore, two aspects require consideration, firstly, the effect of chromatic aberration in modifying colour and, secondly, the effect of colour in changing the apparent magnitude of chromatic aberration.

### *Effect of chromatic aberration in modifying colour of small sources.*

It has been shown in § 4 that when small white and yellow sources are gradually reduced in intensity a point is reached at which it is found impossible any longer to differentiate between them. Now a yellow source owes its colour to the absence of rays of the spectrum of short wave length. These rays have been shown in § 4 to form the largest and least intense aberration discs, and the hypothesis has therefore been advanced that the failure to differentiate between white and yellow sources occurs, when, owing to reduced intensity, the eye is no longer able to recognise the presence or absence of these blue and violet aberration discs, that is, when their intensity is below the threshold of stimulation of the cones. Now under the same conditions it is still found experimentally that red, green and blue sources are at once identified. This may be explained on the above hypothesis, since the presence or absence of the parts of the spectrum concerned is at once observed owing to the smaller and more intense aberration images formed by them being still above the threshold of vision.

This is again confirmed by experiment, for if the intensity of illumination be still further reduced so as to make these discs fall below the threshold value then small white, red, green and blue sources fail to be recognised apart. Under certain conditions of feeble illumination chromatic aberration would seem to be capable, therefore, of modifying the colour of small sources to a considerable extent. In the case of large coloured sources it is found that when the intensity is sufficiently reduced the colour fades, leaving a colourless image. On still further reducing the intensity this also disappears. This difference between the colour and colourless threshold are known as the photo-chromatic interval. Its presence is explained by the different sensitiveness of the cone apparatus which perceives colour, and the rod apparatus which shows form only. It is probable that this phenomenon plays a considerable part in the effect of intensity on the colour of small sources.

### *Colour of extended sources is not modified by chromatic aberration.*

In § 4 a brief description has been given of the way re-combination of rays of different colour occurs to form white light within the image of an extended light source; it is also shown that the coloured fringes at the edge extend within and beyond the geometrical edge of the image for a distance equal to the semi-diameter of the largest aberration disc. In the case of a coloured source it will be seen that re-combination in a similar manner occurs within the image which therefore causes the colour of the image to be the same as that of the source. The absence of certain rays from the spectrum will necessarily modify the aberration fringes. For example, if blue-violet rays are absent the external blue-violet fringe will be reduced in width and the yellow internal fringe will be modified to the same extent; because the overlap of the aberration discs produces the central area of the image. The removal of yellow rays will not affect the width of the yellow internal fringe in the same way that removal of blue-violet rays modifies the external, but the principal effect will be a reduction in the difference of the intensity values on either side of the geometrical image. If red or green rays are absent as, for example, with light from blue-green or violet sources then the modification will chiefly concern the colour of different parts of the fringes; the changes will probably escape notice in comparison with the more definite change in colour of the rest of the image. If the light from the source is deficient in rays corresponding to the middle of the spectrum, and particularly if yellow rays are involved at the same time, then it is found experimentally that the eye suffers from what may be termed focal instability. Take for instance the case of a violet source; it is found that the emmetropic eye focusses first the red rays while the blue rays form a diffuse halo round them; and then after an interval the blue rays form the focussed image, the red image being diffuse. These images alternate irregularly unless by voluntary effort one image be kept constantly in focus. As a rule it is found that one image tends to predominate; it is usually the one that is focussed with the least accumulative effort, or has the greatest brightness. It is not possible to determine how much of the centre of the spectrum must be suppressed for the phenomenon to be witnessed.

Focal instability causes considerable eye strain. This is due in all probability to fatigue of the muscles of accommodation. The same explanation can be offered for the discomfort caused by watching a screen alternately illuminated by light corresponding to rays a considerable distance apart in the spectrum, for example, in the Urban method of colour cinematography (35) red and blue-green images are alternately projected on to the screen which combine physiologically to produce a picture in more or less natural colours. To keep both the red and blue-green images in focus, it is necessary for the accommodation of the eye to be rapidly changed. It appears to be the effort to do this which causes the eye strain.

A method of correcting focal instability would be to render the focal length of the eye the same for both red and blue-green rays. This may be done by placing in front of the eye a correcting lens consisting of a negative flint glass lens neutralised by a convex lens of crown glass. Now assuming the relative dispersive powers of flint and crown glass to be 30 and 50 respectively, the effective dispersive power of the combination is given by the difference of these values, namely 20. Now the equivalent focal length of the eye is 15 mm. and the relative dispersive power 45. To neutralise the chromatic aberration of the eye lenses of approximately 2¼ times the power of the eye would be required, *i.e.* 7 mm. focal length. These lenses, however, would have to be placed at the equivalent focal distance from the retina, namely, 15 mm. Since the smallest distance possible in practice would be approximately 35 mm. the focal lengths of the lenses can be reduced in the same ratio, namely, to 15 mm. A concave lens of flint glass of 15 mm. focal length cemented to a convex crown glass lens of the same power will neutralise the chromatic aberration eye. Such a lens was tested by Helmholtz to ascertain its effect on visual acuity. The improvement in definition was found to be very slight (36).

A practical application of the effects of focal instability has been found in a test for small amounts of astigmatism. In front of an extended source of white light is placed an opaque screen in which has been cut a series of very narrow straight apertures radiating from the centre towards the edge of the disc. In front of this is mounted a sheet of glass transmitting the extreme ends of the spectrum only.

On examining this with the emmetropic eye no difference in the colours of the apertures is to be noticed. If, however, astigmatism is present when the eye has focussed the red rays sharply in the axis of shorter focal length blue rays will be focussed on the axis at right angles. The unfocussed images will produce wide fringes which stimulate the retina, the result being that the apertures appear strongly tinted by the colours that are diffusely focussed. The effect being that the apertures in one meridian appear red, while those at right angles are blue. If the colours used be complementary to one another then it is found that simultaneous contrast tends to accentuate still more the difference in colour of the quadrants. I have found with this test that it is easily possible to detect the presence of 1/10 diopter of astigmatism.

### *Effect of colour on magnitude of chromatic aberration.*

In the previous paragraph the effect of colour in modifying aberration images has been briefly described, in the case of a coloured source on a black background. It remains to consider the case when both source and background are coloured. One example will be considered because similar phenomena may be observed with all colours. Suppose the light from both source and background to consist of two homogeneous radiations of the same intensity, the former red the latter blue. Then if the eye is focussed for red rays the blue will form aberration discs, the centres of which fall along the line of demarcation. A blue fringe will thus be produced half of which lies on either side of the edge of the image formed by the sharply focussed red rays. The intensity curve taken at right angles to the edge will therefore have the following features.

Where the red image falls the intensity everywhere has a uniform value of 100 except for the edge of the image where the blue fringe is formed in addition. Here the intensity rises uniformly from 100 at the edge of the fringe to 150 at its centre. At the point where the edge of the red image is reached the intensity drops abruptly to 50 and from here it rises uniformly to reach the value 100 at the edge of the fringe. The blue image from here continues with uniform intensity of 100. When, therefore, the red rays are in focus the red side of the edge of the image appears to the eye to have three times the intensity of the blue side. Further, the presence of half the blue fringe on the red side of the edge causes the colour of that side to be purple. The colour of the blue side of the edge is normal. If the eye is focussed for blue rays instead of red the converse is the case. For it is the red side of the edge which is of half the normal intensity and the blue side which is violet in colour and of increased intensity. In normal vision involuntary changes in the focus of the eye cause first one image and then the other to form focussed images on the retina. The intensity and colour of the two edges, therefore, appears to alternate. This is the second type of chromatic instability. Its effects are not so obvious as are those of the type previously given. The phenomena just described can be well shown by means of the chromatic telescope. The changes of colour and intensity of the edges being clearly defined.

### 10. THE DIFFRACTION AND RESOLVING POWER OF THE EYE.

A knowledge of the distribution of intensity in the chromatic fringe of a point source provides one of the data that are necessary for calculating the intensity distribution in the images formed on the retina. The additional information concerns the effects of spherical aberration, astigmatism and diffraction at the aperture of the pupil. Diffraction will alone be considered in this paper because the two aberrations can be regarded as abnormal. Diffraction is in a very different position, for it is not caused by any error of the refracting system of the eye, but is due to the nature of light itself; all lenses, however perfect, must show it. It is therefore not an aberration but a property.

*Diffraction.* The effects of diffraction at a circular aperture are well known. For example, in the case of monochromatic light the image produced by a lens is found to consist of a central bright spot surrounded by a number of fine black and white rings. When the source is non-homogeneous the rings will be strongly coloured and the number visible will be less than with a monochromatic source (37). The radius of the first dark ring is given by the formula

$$R = \frac{F \times \lambda \times M}{\frac{D}{2} \times \pi} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4).$$

When $F$ is focal length of lens, $D$ is diameter of pupil or diaphragm and $\lambda$ is wave length of light. $\left(\frac{M}{\pi}\right.$ for the first dark ring will be seen later to equal ·61.$\left.\right)$

The formula shows that the radius of the first dark ring varies directly with the wave length and inversely with the diameter of the pupil. In the case of the other rings the same relationship is found to hold. Decreasing the size of the pupil, which has the effect of diminishing the aberrations of a lens, has the opposite effect on the diffraction pattern.

The approximate size of the radius of the first dark ring in the case of the eye is shown in Table 20.

TABLE 20. Radius of first dark ring with pupils of different diameter, and light of different wave length.

| | Diameter of pupil | 1 mm. | 2 mm. | 4 mm. |
|---|---|---|---|---|
| Extreme red ... | 7600 A.U. | ·014 mm. | ·007 | ·0035 |
| Yellow ... ... | 6000 | ·011 | ·0055 | ·0028 |
| Green ... ... | 5000 | ·0092 | ·0046 | ·0023 |
| Extreme violet ... | 3800 | ·007 | ·0035 | ·0018 |

It will be shown later that the mean radius of the first dark ring for white light is approximately ·01 mm. with a pupil diameter of 1 mm. Assuming as before that the diameter of the retinal cone elements in the fovea is ·0025 mm. diameter, it will be seen that the radius of the first dark ring is equal to eight times the cone diameter with a 1 mm. pupil. The ratio for 2 mm. and 4 mm. pupils being 4 and 2 respectively.

From the point of view of the resolving power of the eye the distribution of intensity in the diffraction pattern is therefore as important as that in the fringe produced by chromatic aberration.

The intensity ($I$) of the different parts of the diffraction pattern can be obtained from Airy's formula (38):

The intensities calculated by this formula are given in Table 21.

$$I=\pi^2R^4\left[1-\frac{1}{2}\left(\frac{m}{1}\right)^2+\frac{1}{3}\left(\frac{m^2}{\underline{|2}}\right)^2-\frac{1}{4}\left(\frac{m^3}{\underline{|3}}\right)^2+\frac{1}{5}\left(\frac{m^4}{\underline{|4}}\right)^2\right]^2 \quad \text{............(5).}$$

TABLE 21. Intensity of diffraction pattern formed by lens with circular aperture.

| | $\frac{M}{\pi}$ | Percentage Intensity $A$ |
|---|---|---|
| Centre of 1st maximum ... | 0 | 100 |
| | ·061 | 98 |
| | ·122 | 86 |
| | ·183 | 70 |
| | ·244 | 54 |
| Half radius 1st dark ring | ·305 | 37 |
| | ·366 | 21·7 |
| | ·427 | 11·3 |
| | ·488 | 4·3 |
| | ·549 | 1·0 |
| Radius 1st dark ring ... | ·61 | 0 |
| „ 2nd maximum ... | ·819 | 1·7 |
| „ 2nd dark ring ... | 1·116 | 0 |
| „ 3rd maximum ... | 1·333 | ·7 |
| „ 3rd dark ring ... | 1·619 | 0 |
| „ 4th maximum ... | 1·847 | ·2 |

It will be observed that $\frac{M}{\pi} = \cdot 61$ for the radius of the first dark ring.

Now beside this type of diffraction caused by a limiting aperture, Helmholtz has also described capillary fringes which he states are produced by diffraction taking place at the indented edge of the pupil. He gives the following reasons for this opinion. If an artificial pupil be formed by punching an aperture in thin metal or cardboard so as to leave an irregular edge, and this be held before the eye, the capillary rays can be clearly seen round a bright source of light. If the card be rotated the capillary rays are seen to rotate at the same time. If, further, a portion of the edge of the card be shielded from light, the capillary rays corresponding to that portion are cut off leaving rays in other quadrants apparently unchanged.

These tests Helmholtz claims show that the capillary rays are produced by diffraction at the edges of the aperture. I cannot convince myself that the fringes are produced in this way for the following reasons: when a distance bright point of light is examined the capillary rays are seen to form a bright coloured fringe round the central image, and the colours are in the order of those of the spectrum. Violet rays are seen to form the most widely separated rays from the central image, while red rays form those nearest to the light source. By placing a screen in front of the eye one can cut off the direct beam and most of the other capillary rays, leaving one or two bright rays isolated. The recognition of the order of the colours in the ray and their identification as complete spectra is then an easy matter. It is not possible for these rays to be produced by diffraction for if they were the opposite order would be found; red rays being most deviated and violet rays least. Secondly, if these were produced by diffraction it would not be possible to cut off the whole of the direct image without at the same time destroying the rays themselves. Thirdly, the intensity of the rays is much greater than would be possible if they were caused by diffraction. Lastly, experiment shows that as the pupil is diminished so the capillary rays decrease in length till with small pupils it becomes difficult if not impossible to detect them. These effects would be impossible on a diffraction basis for one would expect the irregularity in the pupil's edge and therefore the obviousness in the capillary rays to be greater with small pupils, even than with large.

To me the evidence is all in favour of the capillary rays being caused by chromatic aberration; for as the pupil dilates the peripheral zones of the lens system of the eye become exposed to light rays, and therefore considerable refraction occurs. Since in the eye the refraction of the periphery is irregular, the refracted rays do not form a continuous fringe of uniform intensity, but a number of isolated bands which I have called "spectra" in this paper because of their origin. The rotation of the capillaries with the artificial pupil is easily explained because irregularities in the edge of the diaphragm would expose irregularly the peripheral zones of the eye, so that as the diaphragm is rotated the portions of the zones exposed would also rotate and therefore also the capillary rays (spectra) produced by them.

*Resolving power.* Fraunhofer in his study of diffraction, investigated the distribution of light in images produced by plain waves, *i.e.* by waves coming from an infinitely distant source. Sir George Airy extended his work and applied it to the practical problem of the resolving power of the astronomical telescope. The formulæ given above are due to him and from them the resolving power is obtained as follows:

Suppose the centres of two point source images to be a distance apart equal to the radius of the first dark ring, then the intensity of light at different points is shown in Table 22.

TABLE 22.

| | Intensity source *A* | Intensity source *B* | Combined intensity |
|---|---|---|---|
| Centre of source *A*... ... | 100 | 0 | 100 |
| | 98 | 1 | 99 |
| | 86 | 4·3 | 90·3 |
| | 70 | 11·3 | 81·3 |
| | 54 | 27·7 | 75·7 |
| Mid point between sources | 37 | 37 | 74 |
| | 21·7 | 54 | 75·7 |
| | 11·3 | 70 | 81·3 |
| | 4·3 | 86 | 90·3 |
| | 1 | 98 | 99 |
| Centre of source *B*... ... | 0 | 100 | 100 |

The intensity half way between the sources is seen to be 74 %, that is 26 % less than the intensity at the sources themselves. Now this difference of intensity should be observable and therefore the sources should appear resolved.

The formula for the resolving power therefore becomes

$$R = 1{\cdot}22 \times \frac{F \times \lambda}{D} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6).$$

This formula is identical with that given by Helmholtz(44) and is at variance with that given by Drude(47). Drude's formula is in error as Gullstrand(45) and Schuster(46) have pointed out through the insertion of the refractive index of the ray in aqueous humour and lens substance instead of in air.

In the case of the microscope Abbe(39) has shown that a different method of calculating the resolving power has to be employed when periodic structures are considered, since diffraction by the object then becomes the important process in place of diffraction by the aperture of the optical system. Now Abbe's theory stated that for resolution of a periodic structure to occur the direct beam illuminating the structure and the two first order spectra on either side of it must be passed by the optical system. When the direct beam itself passes obliquely through the system, one first order spectrum is alone sufficient. Abbe showed by experiment that the same method of calculation must be applied to the case of the telescope when periodic structures are being examined, and his theory showed that other optical instruments are affected in a similar manner.

The method of calculating the resolving power is not therefore determined by any property of the optical system but by the type of object examined. If there is phase relationship between different parts of the object then the Abbe theory must apply, but if the object consists of separate sources each of which is independent then the Fraunhofer method of determining resolving power must be used. Now the images presented to the eye are of both kinds so that the resolving power available should be determined on both theories. That obtained on the Fraunhofer basis can be readily obtained from the formula given above, namely:

$$R = 1{\cdot}22 \times \frac{F \times \lambda}{D} \quad \text{..........................................(6).}$$

If $F = 15$ mm., $\lambda = 6000$ A.U. and $D = 2$ mm., then $R$ is found to equal ·0055 mm.

On Abbe's basis the calculation can be made as follows. For resolution to occur the direct beam and the first order spectrum on one side must both be able to enter the pupil.

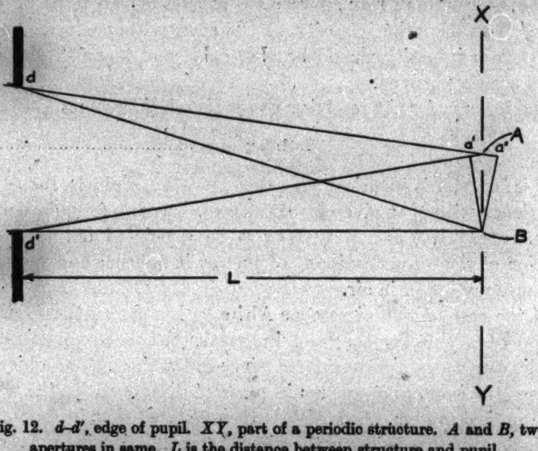


Fig. 12. *d–d'*, edge of pupil. *XY*, part of a periodic structure. *A* and *B*, two apertures in same. *L* is the distance between structure and pupil.

If in Fig. 12 *A* and *B* are two elements of a periodic structure *XY* and *d–d'* is the pupil. The direct beam enters close to *d* and the diffracted beam close to *d'*. Then the distance *bd* minus the distance *ad* is greater than the distance *bd'* minus the distance *ad'* by one whole wave length. Along *ad'* mark a point *a'* such that *a'd'* equals *bd'*; similarly produce *ad* and mark a point *a''* such that *a''d* equals *bd*. Then it is clear that *a'a''* equals λ.

But from similar triangles $\frac{dd'}{L}$ equals $\frac{\lambda}{ab}$ where *L* is the distance between the eye and the object.

But $\frac{L}{AB}=\frac{F}{R}$ where $F$ is the focal length of the eye (distance between retina and posterior nodal point) and $R$ is the resolving power.

Therefore by substitution $$R=\frac{F\times\lambda}{D}\qquad\ldots\ldots(7).$$

If as before $D=2$ mm. and $\lambda=6000$ A.U. and $F=15$ mm. then $R$ is found to equal ·0045 mm.

On the Abbe basis a value of ·0045 mm. is obtained while the Fraunhofer basis gives the value ·0055 mm. This discrepancy requires further investigation.

Formula (6) which has been since the time of Airy accepted as giving the resolving power of the telescope, would not appear on further examination to give the limit to the resolving power. This would occur when the intensity midway between two sources is equal to the intensity at the sources themselves. Calculation shows that this occurs when the distance between the sources is equal to ·82 times the radius of the first dark ring. The value found above on the Fraunhofer basis (·0055 mm.) has therefore to be multiplied by ·82 in order to obtain the limiting value for the resolving power. This gives ·00451 as the limit, *i.e.* the same value as was obtained on the Abbe basis. The formulæ for the resolving power on the two theories become identical (Formula 7).

Now this is a fact of great interest because in the case of microscopic optics it has been held that the Abbe and Fraunhofer theories were rivals. One of the differences has been the discrepancy between the values of the resolving power of the same optical system given on the two hypotheses. The agreement now obtained between the corrected formulæ not only suggests that other differences may be more apparent than real but also that the two theories may be but different aspects of one and the same physical process. Or alternately the Abbe theory may be one special case (applicable in the case of the microscope when critical illumination is being used) of a more general theory of which the Fraunhofer theory forms a basis.

*Resolving power of the eye.* The values for the resolving power of the eye just given are calculated from the diffraction theory on the assumption that the eye is a fully corrected lens system.

Assuming spherical aberration and astigmatism to be absent the eye still suffers to a considerable degree from chromatic aberration. Further, the new value ·0045 mm. is for a pupil of 2 mm. diameter and for light of 6000 A.U. wave lengths. The value for white light will be different and may be obtained approximately by making a summation of the individual waves present, assigning to each a value according to its physiological luminosity as explained in § 4. Calculation shows the value to be approximately the same as that for the yellow rays. Now not only are rays of approximately 5800–6000 the ones which form sharp images on the retina according to my hypotheses, but they are also the rays of greatest luminosity in the spectrum. One would appear justified therefore in assuming the values for white light to be closely those for yellow rays. I will therefore assume that this is the case for the purpose of calculation. Approximate values for the intensity of illumination on the different retinal cone elements caused by the image of a point source formed by the eye, will now be given, diffraction and chromatic aberration being both taken into consideration.

TABLE 23. Percentage intensity of illumination falling on cones with different diameters of pupil.

| Pupil diameter | Central cone | 1st row | 2nd row |
|---|---|---|---|
| 2 mm. | 15·4 % | 7·7 % | 1·2 % |
| 3 „ | 18 | 6 | 1·4 |
| 4 „ | 25·4 | 5·6 | 1·7 |

Now if two point sources are such a distance apart that their centres correspond with the centres of two cones which are separated by an intervening cone, then the intensity of illumination falling on the cones is at once obtained from the above figures. For example, in the case of a 2 mm. pupil the intensity stimulating the cones corresponding to the images is 16·6 while the intervening cone receives 15·4. There is therefore 7 % less light on the middle cone. With a 3 mm. pupil the difference in a similar way is found to be 38 % and with a 4 mm. pupil 58·6 %. With a 2 mm. pupil resolution of the two sources should be just possible and the above figures show that the facility should rapidly increase with increase of aperture. Under ordinary circumstances the benefit of pupils of larger diameter than 3 mm. is detracted from by the spherical aberration and astigmatism that are simultaneously introduced.

A limit to the size of the pupil that can be usefully employed is also set by the following considerations: According to Lord Rayleigh, in telescopic optics a limit is set to the aperture of the lens system by the fact that the waves that reach the same part of the image after traversing paths of different length through the lens system interfere (in the optical sense) with one another in such a way as to cause the definition to be worse if the aperture of the system exceeds the value given by the following equation:

$$\text{semi-aperture in radians} = \left(\frac{\lambda}{F}\right)^{\frac{1}{4}} \text{ where } F \text{ is the equivalent focal length of the system.}$$

If this reasoning be applied to the eye, it is found that the semi-aperture must not exceed ·079 radian for the best definition. This corresponds to a pupil diameter of 3·00 mm. The resolving power of the eye would therefore appear to be greatest for pupils between 2·5 and 3 mm. in diameter. Let it be assumed for the sake of argument that the optimum is at 2·8 mm., then the corresponding value for the difference of intensity is found to be 32 %.

It would appear of interest to calculate the limiting value of the resolving power and to compare it with the value given by the diffraction theories and with the experimental value. The limit is clearly reached when there is no difference of intensity between the points of the retina on which the images fall and a point intermediate between them; that is found to be when the distance between the centres of the images is ·0035–·003 mm. The value obtained by formula (7) above for the limit of resolution gives the value ·0032 mm. Parsons(40) gives a much lower value for the acuity with a pupil of 2·8 mm., namely, ·0025 mm.; it is not stated how this value was obtained but I find that it corresponds to that given by Drude's formula. Since Gullstrand and Schuster have shown this formula to rest on a faulty basis, this value for the acuity given by Parsons would also appear to be inaccurate.

*Experimental determination of resolving power.* This has been carried out by a number of observers by a variety of different methods. The following examples may be quoted.

Hooke and others using the distance between the centres of point sources as a measure of the acuity, obtained a value of approximately ·0042 mm. Aubert, using the distance between the edges of two white squares on a black ground, obtained the value ·0025 mm. Helmholtz, using a wire grating, obtained the value ·0045 for the distance between the centres of the bars (which were equal in width to the spaces between them), that is ·00225 for the distance between the edges of the spaces. Cobb(41) by a similar method obtained the value ·0045 mm. Lastly, the probable mean value found by the microscope for the diameter of the foveal cones is ·0025 mm. This itself places a limit on the fineness of structure that may be resolved. These values are shown in tabular form below to facilitate comparison.

If a mean be taken between the experimental determinations given at 5, 6, 7 and 8 in the table the value thus obtained (·003) agrees well with the resolving powers calculated from the theories of diffraction (·0031), also with that calculated by me from the intensity distribution in the image due to chromatic aberration and diffraction; ·003 to ·0035,

TABLE 24. Resolving power of eye.

| | | |
|---|---|---|
| 1. | On Abbe theory $\lambda=5800$, $D=2{\cdot}8$ mm. | ·0031 mm. |
| 2. | On Fraunhofer theory $\lambda=5800$, $D=2{\cdot}8$ mm. | ·0031 mm. |
| 3. | Calculated from chromatic aberration and diffraction | ·0025–·0035 mm |
| 4. | From size of cones of retina | ·0025 mm. |
| 5. | Found by grating experiments (Helmholtz) | ·0023 mm. |
| 6. | Found experimentally, two sources (Hooke) | ·0040–·0045 mm. |
| 7. | Found experimentally by Cobb | ·0045 mm. |
| 8. | Aubert | ·0025 mm. |

the inference being that the presence of chromatic aberration has little or no effect on visual acuity.

This deduction is itself confirmed by the experiments of Helmholtz on rendering the eye achromatic. He found that this caused little or no increase in visual acuity. This fact can I think be satisfactorily explained by an examination of the form of the curve for the distribution of light in an image produced by a lens which suffers from chromatic aberration and astigmatism. The tip of the curve is found to be strongly convex for a short distance and then to change to a concave curvature which gradually approaches the base line. If a straight line is drawn from the top of the curve to the point where the change in the form of curve occurs, then the line produced meets the curve again at a point as far away from the "point of change" as the top of the curve. When two such curves overlap, therefore, and the interval between their apexes is gradually decreased then resolution is found to cease when the two points of change meet. This interval is found to be the same as that at which resolution ceases when diffraction alone is considered. This would appear to confirm the results of experiment that chromatic aberration has no deleterious effects on visual acuity.

The question as to the best pupil diameter for the greatest visual acuity has been investigated experimentally by several observers; probably the most accurate determinations are those that have been made by Cobb. Using artificial pupils and a grating test object he found that with a 1 mm. pupil the experimental resolving power of the eye was somewhat higher than the physical formula $\theta = 1{\cdot}22\,\frac{\lambda}{D}$ would require to be given. As I have pointed out above, this formula requires modification to $\theta = \frac{\lambda}{D}$. The theoretical resolving power is then found to be nearly 10% higher than that which Cobb(41) found in practice. This is what one would expect to be the case since the eye suffers from chromatic and other aberrations.

The experimental value obtained by **Cobb** for the resolving power, namely ·0045 mm., is not so high as the mean value obtained by the other experimenters mentioned in Table 24. Two reasons may be advanced for this: Firstly in **Cobb's** experiments the test object consisted of a group of bands, which varied alternately from maximum to minimum, the transition from one to the other being a gradual one. In the usual tests for visual acuity the transition is abrupt, and would therefore tend to give higher values for resolving power. Secondly, the optimum diameter for the artificial pupil was found to be 4 mm., and this was placed at a distance of 15 mm. from the eye. Calculation shows that the angle of the cone of rays passed by the diaphragm and falling on any one part of the retina is practically the same in its effects as that of a natural pupil of 2 mm. diameter. This would give a value for the resolving power of ·0044 mm. which is almost in agreement with **Cobb's** value of ·0045.

It remains, therefore, to consider why he found so small an effective aperture to give the optimum resolving power. This may have been partly due, at all events, to the peculiar intensity distribution in the test object; or it may have been caused by aberrations introduced if wider apertures were used. The three observers for whom values are given by **Cobb** were obviously not chosen for their keenness of vision; one had 1·75 diopters of astigmatism. It is not surprising, therefore, that higher values for the resolving power coupled with greater diameters for the optimum pupil diameter were not obtained.

A further point to be noticed in the values for resolving power in Table 24 is that the mean of the experimental values falls short of the limit apparently set by the diameter of the cone. It is difficult, as **Parsons** has pointed out, to make any definite statement on this point so long as the position at which stimulation occurs is unknown. If, however, we assume that between the cones there is as little as ·0003 mm. of intercellular cement substance, then the interval between one cone and the one after the next is ·0031 mm., which agrees closely with the other values for the resolving power.

Even if we assume, however, that the agreement is not so exact as this, the interesting fact is still evident that the retinal structures has almost exactly the fineness which the maximum resolving power, given by the refracting system of the eye, would demand.

*Practical applications.* The suggestion obtained from the above calculations of the resolving power, namely, that the optimum pupil diameter is 2·8 mm. approximately introduces several points of practical nature. Firstly, it is clear that if for any reason the diameter of the pupil of the eye is restricted to less than 2·8 mm., not only is light lost but visual acuity is decreased also. Similarly, if the pupil is much greater than 2·8 mm. then again definition suffers by the introduction of aberrations.

This would appear to explain why the greatest acuity is not reached with very bright or very dull light. An artificial restriction of the pupil or abnormality in the pupilomotor mechanism would have similar effects. Since an artificial restriction to the diameter of the bundle of rays entering the eye occurs when certain optical instruments are used, such as the microscope and telescope, the question as to the size of the exit pupils (Ramsden discs) of such instruments is introduced. In very few cases do the Ramsden discs of such systems equal 2·8 mm. The Gallilean telescope system is of course an exception.

Microscopists have learned to recognise what is called empty magnification, that is magnification which decreases the illumination of the field and does not at the same time increase definition. The facts are not, however, I think, sufficiently appreciated by designers of other optical instruments. For maximum definition, the practice that should be adopted appears to be to restrict the aperture of the pencils of light entering the eye to 2·8 mm., to so adjust the depth of the eyepiece cup that the eye is placed so that the plane of the exit pupil (Ramsden disc) coincides with the true plane of the iris of the eye, and then to adjust the intensity of the field that the observer's pupil shall open to a slightly greater amount. As the field is seldom as bright as objects seen outside the instrument, light entering the periphery of the eye should be as far as possible excluded, in order that the maximum acuity may be realised (43).

Another important application relates to the correction of chromatic aberration in optical instruments. In practice two corrections are made, (1) in order to bring to a focus in the same plane rays of different wave length (correction for chromatic difference of focus), (2) in order that the pictures produced by rays of different wave length shall be of the same size (correction for chromatic difference of magnification).

The conclusion reached in this paper, namely, that chromatic difference of focus has little effect on definition would point to this correction being of much less importance than the elimination of chromatic difference of magnification. In the case of chromatic difference of focus one would not expect the definition to be obviously affected even if the coloured rays were brought to foci which were further apart than they normally are in the unaided eye. With chromatic difference of magnification entirely different conditions are to be found, for as I have shown in § 8 as chromatic difference of magnification increases the definition improves up to a certain point, and then suddenly breaks down to reach a very low value. This breakdown usually occurs at a point corresponding to the periphery of the fovea and is not therefore a detriment to normal vision. When, however, an optical instrument is used which increases the amount of chromatic difference of focus, this breaking down of the definition may occur at a point which corresponds to the fovea, and the effects may then seriously affect the sharpness of the image. This conclusion was confirmed by the following experiment: a telescope of 2 diameter magnification was constructed of three simple crown glass plano-convex lenses, the first lens forming an objective, and the other two a Huygenian eye-piece system. The eye-piece lenses were placed at such a distance apart that chromatic difference of magnification was corrected. The definition of the image through this telescope was found to be quite good, and yet calculation showed that the chromatic difference of focus of the rays on the eye was increased between two and three times. Another telescope was made in which the magnification was 10. The objective again consisted of a simple crown glass lens, and a compensating eye-piece was employed which corrected the chromatic difference of focus. The definition of the image formed by this instrument was quite good, although there were coloured borders to the contours which theory would demand. In the low power telescope objective, therefore, it is usually sufficient if correction for chromatic difference of magnification be considered, because such a formula will give as a rule sufficient correction for chromatic difference of focus. In high power telescopes and in the microscope both must be corrected independently. The practice in microscopy of correcting chromatic difference of magnification in the virtual image formed by the objective, by means of compensating eye-pieces, has the defect that only one degree of correction can thus be introduced; which may be either more or less than is required. Holoscopic eye-pieces, in which the amount of correction can be adjusted, suffer from defects so serious that they receive very little use except for objective testing. I have recently however constructed a new type of eye-piece which gives adjustable compensation, and does not at the same time suffer from similar disadvantages. The details of this eye-piece are to be described elsewhere.

I wish to take this opportunity of thanking Mrs S. McIntosh for her very valuable assistance with the translations of some of the foreign journals referred to previously in this paper.

## SUMMARY.

*Section* 1. A *résumé* is given of the various hypotheses that have been advanced to explain how it is that the detriment to vision caused by chromatic aberration is so slight. Helmholtz outlined the hypothesis which fitted in best with all the data then available; details were, however, lacking and on certain points I have found his hypothesis to be at fault.

*Section* 2. Helmholtz calculated the diameters of the aberration discs produced by rays of different wave length, assuming the refractive indices of the eye media to be the same as those of water. Experiment shows these values to be too low. From the evidence available I have calculated the probable values of the refractive indices.

*Section* 3. According to Helmholtz the focal planes of rays of different wave length are so adjusted in relationship to the retina that the red and violet aberration discs are of equal diameter. Calculation shows that when this is the case the retina coincides with the focal plane of green rays (5000 A.U.). This I find by experiment is not the case in normal vision, the focal plane of yellow rays (5800 A.U.) being usually selected.

The diameter of aberration discs formed by rays of different wave length have therefore been re-calculated, using the corrected values for the refractive indices. The selection of the focal plane for yellow rays in preference to green rays would appear to have certain advantages. This deduction is I find confirmed by experiments with an uncolour-corrected telescope, and also by calculations which show that a greater proportion of the light forms the centre of the image on my basis than it does on that of Helmholtz.

*Section* 4. In discussing the distribution of intensity in the plane of the retina Helmholtz considered the case of an extended source of white light only, and he did not obtain confirmation of his deductions by experiment. I have made good this omission extending similar treatment to the case of small sources.

I have also calculated for these cases the probable distribution of light on the separate cones of the retina. The values thus obtained show features of considerable interest. The following case may be quoted as example. In the image of a point source with a pupil of ·9 mm. over

76 % of the incident light falls on one cone; while the cones round it receive only 3 %. If the intensity of light stimulating the central cone is equal to 100 that falling on the surrounding cones is less than 4 %. It is thus that visual acuity remains unaffected by chromatic aberration, and the disappointing results of achromatising the eye are therefore explained.

*Section* 5. The effects of colour mixture on the images presented to the eye as a result of chromatic aberration were not considered by Helmholtz. My calculations show that this factor is of considerable importance. For example, the image of a point source of white light consists of a bright central nucleus of yellow rays surrounded by a diffuse blue-violet fringe of feeble intensity. In the case of a point source of yellow light the bright yellow nucleus is alone present.

The ability to differentiate between point sources of yellow and white light therefore depends on the power to recognise the presence or absence of the blue-violet fringe. It was anticipated that this would depend on the intensity of the image, differentiation being possible in bright light and impossible in feeble. Experiments show that this supposition is verified.

Now this raises the following very important question if the presence or absence of the blue-violet fringe is appreciated, how is it that the sharp image alone reaches consciousness as has been postulated in Section 4?

The simultaneous differentiation between small white and yellow sources and the suppression of the blue-violet fringe can be explained as follows:

If two images, one sharp and one diffuse, be simultaneously presented to the retina, the sharp tends to cause suppression of the diffuse. In spite of this any alteration in the diffuse image is at once appreciated, which shows that the diffuse image stimulates the organ of vision although it may not be reaching consciousness. Although the blue-violet fringe image is not perceived, therefore, its presence or absence is notified to the higher centres and the colour of the source interpreted as white or yellow accordingly.

*Section* 6. A number of factors reduce the effects of chromatic aberration of the eye; two of them were described by Helmholtz, namely, (1) the greater luminosity of rays corresponding to the middle of the spectrum, and (2) effect of size of aberration discs on intensity. Other factors of importance are (3) the shortened spectrum of ordinary light sources, (4) the order of colours in the spectrum, (5) the position in the spectrum of rays forming sharply focussed images on the retina, (6) the variation of dispersion with wave length of the eye media, (7) the shape of the pupil, (8) the effects of simultaneous contrast, (9) the antagonism between focussed and unfocussed images, (10) the effect of threshold, (11) chromatic difference of magnification.

All the above factors are shown to reduce the effects of chromatic aberration.

*Section* 7. The conditions of night vision reduce considerably the effects of chromatic aberration. The following changes are found to occur:

(1) The change in the intensity distribution causes the aberration image to be 20–30 % sharper than in day vision.

(2) Colour disappears from the fringes and the point of maximum brightness moves into the green, the latter causes a reduction in the amount of diffraction caused by the pupil.

*Section* 8. Gullstrand pointed out that the eye should suffer from chromatic difference of magnification. He advanced two hypotheses for its non-observance. Neither of these are found by me to fit in with the facts. I have obtained an alternative explanation by calculations of the distribution of intensity, and I find that under certain conditions the definition of the image on the fovea may be actually improved 15 % by the phenomenon. Chromatic stereoscopy investigated experimentally by Donders was incorrectly explained by Einthoven. I have advanced a more probable view which draws a close union between chromatic stereoscopy and chromatic difference of magnification.

*Section* 9. With regard to coloured objects two aspects require investigation: (1) the effect of chromatic aberration on colour, (2) the effect of colour on chromatic aberration. The former has been already partly considered in Section 5, the latter produces an effect which may be called chromatic focal instability. The conditions under which it occurs and the causation of eye strain by it are considered. A test for small amounts of astigmatism can be obtained by applying the principles described above.

*Section* 10. The resolving power of the eye given by experiment is found to vary between ·0023 and ·0045 mm. The variation is due to differences in the type of test object, the intensity of the light, the size of the pupil and the keenness of sight of the observer. Thus Cobb obtained somewhat low values for the resolving power; these were obtained with an effective pupil of 2 mm. diameter and light of medium intensity.

The value of the resolving power can also be obtained by calculation. In this, chromatic aberration and diffraction are the important factors. With regard to diffraction the Abbe and Fraunhofer theories appear to give different values; I have, however, been able to eliminate this difference.

The optimum resolving power would appear to be about ·0031 mm., which should be obtained with a pupil of not less than 2·8 mm. diameter. This value fits in well with experiment and also with the limit set by the fineness of the structure of the retina. A pupil smaller than 2·8 mm. gives a lower value for the resolving power because of diffraction at the aperture of the pupil. A pupil larger than 3 mm. on the other hand is not beneficial because of the high degree of spherical aberration possessed by the more peripheral zones of the eye, and because of the destructive interference which as **Rayleigh** has shown is introduced by large apertures. Certain practical applications would appear to follow from these considerations as to the diameters of the exit pupils of optical instruments, and as to the chromatic correction.

## REFERENCES.

(1) **Newton.** Optics, Book 1, Part II, prop. 8.
(2) **Euler.** Journ. Encyclop. 1765, **2.** p. 146.
(3) **Dollond.** Phil. Trans. **79.** p. 256.
(4) **Wallaston.** Phil. Trans. 1801, **1.** p. 50.
(5) **Young.** Phil. Trans. 1801, **1.** p. 50.
(6) **Fraunhofer.** Gilbert's Annalen, **56.** p. 304.
(7) **Matthiessen.** Comptes Rendus, **24.** p. 875.
(8) **Helmholtz.** Physiol. Optics, 3rd Edition, p. 146. 1909.
(9) **Wolf.** Wiedemann's Ann. **32.** 1888.
(10) **Einthoven.** Arch. f. Physiol. **63.** p. 166. 1896.
(11) **Ganot's** Physics, Edition xi, p. 561. 1882.
(12) **Hermann.** Hdb. d. Physiol. **3.** p. 99.
(13) **Thompson.** Optical Tables, 1905, p. 27.
(14) **Tscherning.** Physiol. Optics, 1904, p. 28.
(15) **Hartridge.** Proc. Roy. Soc. B. **86.** p. 128. 1913.
(16) **Abney.** Researches in Colour Vision, p. 94. 1913.
(17) **Houston.** Proc. Roy. Soc. A. p. 424. 1916.
(18) **Parsons.** Roy. London Ophthal. Hosp. Reports, **19** p. 110.
(19) **Parsons.** Ibid. p. 109.
(20) **Parsons.** Colour Vision, p. 34. 1915.
(21) **Parsons.** Roy. London Ophthal. Hosp. Reports, **18.** p. 253.
(22) **Stokes.** Collected Papers, **4.** p. 55.
(23) **Parsons.** Colour Vision, p. 36. 1915.
(24) Rheinburg Quekett Journal, Nov. 1900.

(25) **Hartridge.** Proc. Physiol. Soc. 1912, p. xxix. (Journ. Physiol. 45.)
(26) **Edridge Green.** Colour Blindness, p. 58.
(27) **Hartridge.** Journ. Physiol. 50. p. 47. 1915.
(28) **Haycraft.** Journ. Physiol. 22. p. 126. 1897.
(29) **Gullstrand in Helmholtz,** Physiol. Optics, Edit. 3, p. 158. 1909.
(30) **Rivers in Schäfer's** Text Book of Physiol. 2. p. 1144.
(31) **Donders.** Accommodation and Refraction of the eye, p. 161. 1864.
(32) **Einthoven.** Arch. f. Ophth. 31. p. 211. 1885.
(33) **Parsons.** Roy. London Ophthal. Hosp. Reports, 18. p. 252.
(34) **Tscherning.** Physiol. Optics, p. 31. 1904.
(35) **Lindsay Johnson.** Photography in Colours, p. 203. 1916.
(36) **Helmholtz.** Physiol. Optics, 3rd Edit. p. 157.
(37) **Preston.** Theory of Optics, Section 163, p. 288.
(38) **Airy.** Camb. Phil. Trans. p. 283. 1834.
(39) Cf. **Conrady.** Journ. Roy. Micr. Soc. 1904, p. 610 and 1905, p. 541.
(40) **Parsons.** Roy. London Ophthal. Hosp. Reports, 19. p. 271.
(41) **Cobb.** American Journ. Physiol. 36. p. 335. 1915.
(42) **Rayleigh.** Cf. **Thompson,** Optical Tables, p. 100.
(43) **Parsons.** Roy. London Ophthal. Hosp. Reports, 19. p. 376.
(44) **Helmholtz.** Physiol. Optics, 3rd Edit. p. 167. 1909.
(45) **Gullstrand in Helmholtz.** Physiol. Optics, 3rd Edit. p. 374. 1909
(46) **Schuster.** Introduction to the Theory of Optics. 1904.
(47) **Drude.** Lehrbuch der Optic. 1906.

## ADDENDUM

A Paper on the resolving power of the eye by **J. J. Lister** occurs in the *Journal of the Royal Microscopical Society*, 33. p 34, 1913. This has come to my notice too late to be considered here, but I hope to give an account of it later.

# ON THE STIMULATION AND PARALYSIS OF NERVE CELLS AND NERVE ENDINGS. Part II[1]. PARALYSIS BY CURARI, STRYCHNINE AND BRUCINE AND ITS ANTAGONISM BY NICOTINE. By J. N. LANGLEY, F.R.S.

(*From the Physiological Laboratory, Cambridge.*)

It was found by Dickinson and myself (1890)[2] that curari, strychnine, and brucine paralysed the pre-ganglionic fibres of the cervical sympathetic of the rabbit, but did not paralyse the post-ganglionic fibres. A similar effect of curari was mentioned by Anderson and myself on the fibres running to the ciliary ganglion of the cat (1892), and to those running to the inferior mesenteric ganglia and the ganglia of the pelvic plexus (1895). These results tended to show that all three poisons had, like nicotine, a paralysing effect on pre-ganglionic nerve fibres in general. I showed later (1901) that the stimulating action of nicotine, which preceded the paralysis, occurred after degeneration of the pre-ganglionic nerves, *i.e.* that nicotine acted directly on the peripheral nerve cells. The fact established a presumption that curari, strychnine, and brucine paralysed the pre-ganglionic nerves by a direct action upon the nerve cells. Later again (1911) I showed that this was the case as regards curari and the nerve cells in the course of the sacral nerves supplying the bladder, since a given amount of curari prevented the contraction normally caused by a given small amount of nicotine, but did not prevent contraction from being obtained by a larger amount of nicotine.

Whilst the fact that curari, strychnine, and brucine in sufficient amount paralyse peripheral nerve cells has in general been accepted, the bearing of this fact upon the theories as to the mode of action of the poisons has passed unnoticed. This applies especially to the theories of the mode of action of curari and of strychnine when they cause a fall of blood pressure, of curari in causing secretion of saliva, and of curari and nicotine in influencing the 'pseudo-motor' affect of the chorda tympani upon the tongue. These theories I propose to consider in subsequent papers.

---

[1] Part I. This *Journal*, 27. p. 224. 1901.

[2] The references are given at the end of the paper in the order of the year of publication.

The experiments on which this paper is based were made for the most part in 1901 on animals anæsthetised first with chloroform, then by A.C.E. mixture administered by a tracheal tube, sometimes with the addition of urethane. The amount of the anæsthetics required to maintain complete anæsthesia was determined in control experiments. I made brief reference to the results in 1911[1]. I have returned to the action of poisons paralysing peripheral nerve cells partly in order to see whether any means could be found of permanently raising the low blood-pressure they produce. All recent experiments on curari have been made on decerebrated animals. The experiments were on the cat.

*Use of term paralysis.* I use, now as earlier, the term 'paralysis of an efferent nerve' to signify that excitation of the nerve no longer has a peripheral effect. This use is in general accordance with the established clinical use of 'paralysis' for loss of function. Similarly, I use 'paralysis of nerve cells' to signify that the nerve cells no longer transmit nerve impulses and that their ceasing to do so is not due to an alteration of any part either of the pre-ganglionic or of the post-ganglionic fibres. As an hypothesis I take it that the paralysis is one of a receptive substance differentiated from the rest of the cell substance, but there seems to me no advantage in using the terms of this or any other hypothesis.

Recently Burns and Watson (This *Journal*, 42. p. 94. 1918) speak of guanidin as poisoning synapses like nicotine, and of atropine as paralysing terminal ganglia. The change in nomenclature seems to me unnecessarily confusing. Nicotine blocks impulses proceeding from pre-ganglionic fibres; atropine blocks impulses proceeding from post-ganglionic fibres. The facts are expressed most simply by saying that nicotine paralyses pre-ganglionic fibres, and atropine paralyses post-ganglionic fibres.

## PARALYSIS OF PERIPHERAL NERVE CELLS BY CURARI, STRYCHNINE AND BRUCINE.

The degree of paralysing effect of curari, strychnine and brucine was determined on the cardio-inhibitory fibres of the vagus, on the secretory fibres of the chorda tympani and on most of the fibres of the cervical sympathetic, viz. those causing dilatation of the pupil, retraction of the nictitating membrane, opening of the eye and secretion of saliva. A few observations were made on pilo-motor nerves in the cervical sympathetic and elsewhere. The effect of the vaso-constrictor fibres was also observed. But in the nature of the case a slight effect is less easily seen than a slight effect on the eye, or on secretion. Moreover as a rule most attention was paid to the effects on the eye. In consequence there may have been a trifling contraction of blood vessels in some of the cases in which I speak of the cervical sympathetic as being completely paralysed.

---

[1] This *Journal*, 43. p. 150, footnote.

A tracing of the blood-pressure was taken. The solutions were usually injected into the femoral vein sometimes into the jugular. Various effects immediately following injection were noted; these for the most part will be dealt with in later papers. The blood-pressure was usually taken from the femoral artery, sometimes from the carotid.

If either the carotid artery or the jugular vein is tied, the pupil on that side enlarges, and its response to drugs is slower than on the opposite side. If both femoral artery and vein are tied, curari only slowly paralyses the muscles supplied by them, and strychnine may cause contraction in the leg.

Paralysis of pre-ganglionic nerves greatly reduces the blood-pressure, and the decreased circulation and insufficient oxygenation if protracted cause a progressive decrease of irritability of all the tissues. But the maximum amount of a paralysing drug which I have injected did not prevent adrenaline, pilocarpine, or stimulation of sympathetic post-ganglionic nerves, from causing their usual effects.

One of the difficulties of stating the amount of curari, strychnine, or brucine required to paralyse any given nerve is that the paralysis with the minimal dose is very brief, since a certain concentration of the alkaloid is necessary to maintain the paralysis. Three minutes after injection, stimulation of a nerve may have no effect, yet a slight effect may be produced by stimulating three minutes later. In my experiments the stimulation was usually about three minutes after the end of the injection of the paralysing drug.

The paralysing effect of drugs varies in different individuals of the same species and is not strictly proportional to the amount given per kilo of body weight. The ease of paralysis is increased by increasing the anæsthetic, especially chloroform. It is also increased by fatigue, and an apparent inequality in the action of a drug may be caused by unequal stimulation. The number of fibres must also be taken into account; with increasing block, an absence of obvious effect on injecting a drug is obtained earlier when there are few than when there are many nerve fibres. Possibly this may account, in part at any rate, for the earlier paralysis by nicotine of the secretory fibres of the sympathetic than of those of the chorda tympani which I found in early experiments (1890).

It must also be borne in mind that usually whilst a certain amount of a paralysing drug will reduce the effect of nerve stimulation to small dimensions, a much larger quantity must be given to abolish the effect altogether. For these reasons I have not attempted to determine, except in a very approximate manner, the amount of a drug required to paralyse the several nerves. Moreover the curari solution was not made up for each experiment.

In the cervical sympathetic there are sometimes nerve cells in the upper part of the nerve which send nerve fibres chiefly to the nictitating membrane and eyelids. In consequence the nerve should be stimulated low in the neck, and in any experiment in which the paralysis of the nerve is unusually delayed, the nerve should be examined microscopically for nerve cells.

### *Action of curari in increasing dose.*

Many autonomic nerves have been described as being paralysed by curari. The occurrence of paralysis has in most cases been contested. This has arisen mainly from the results obtained in one vertebrate being taken as applying to all. In fact the paralysing effect of curari, and of other drugs, is very unequal in different animals, so that a given amount may cause paralysis of an autonomic nerve in one animal and not in another. Even with skeletal nerves there is sometimes considerable difference, thus Camis (1915) has shown recently that 20 times as much curari is required to paralyse motor skeletal nerves in the Argentine frog as in European frogs. A subsidiary cause of divergence of result in some cases has been a lack of recognition of the short duration of paralysis with minimal and moderate doses.

Boehm (1875) considered that the absence of vagus paralysis by curari found by various observers was due to their using a weak preparation. The differences described in the relative time of paralysis of the vagus and of skeletal nerves is too great to be satisfactorily explained in this way. Since there is at present no evidence of the presence in curari of two substances paralysing with unequal ease the vagus and skeletal nerves, I attribute the differences found mainly to unequal susceptibility in the different animals used.

An analysis of the accounts shows that nearly all the mammalian nerves said to be paralysed are pre-ganglionic nerves. In the frog paralysis of the vaso-motor nerves in the sciatic has been described and these are post-ganglionic. In fact in the frog the post-ganglionic nerves are not exempt from paralysis by curari as they are in the bird and the mammal.

Cl. Bernard (1856) found that curari in excess of that required to paralyse skeletal motor nerves, paralysed the cardio-inhibitory fibres of the vagus, and the secretory fibres of the chorda tympani. He stated also that after section of the cervical sympathetic in the rabbit, curari did not cause a rise of temperature of the ear[1], this he regarded as due to paralysis of the sympathetic. Kölliker (1856) obtained similar effects on the vagus and chorda tympani, and also on the pupillo-dilator fibres in the rabbit and the vaso-motor fibres of the frog's sciatic. v. Bezold (1863) observed that after a large amount of curari, stimulation of the spinal cord in rabbits had little or no effect on the heart beat or on blood-pressure; this he attributed to paralysis of the exciting (accelerator) nerves of the heart. Eckhard (1873) stated that when the skeletal motor nerves of the rabbit were paralysed by curari, stimulation of the spinal cord caused little or no increased blood flow from the cut penis. v. Frey (1877) observed that curari in amount just sufficient to paralyse the nerves of skeletal muscle greatly decreased the effect of the chorda tympani upon the blood flow through the sub-maxillary gland. Feotistoff (1889) found that certain samples of curari paralysed the cardio-inhibitory fibres of the vagus, not after the motor nerves but at practically the same time; the paralysis being brief in the rabbit and dog and long lasting in the cat. According to Tillie (1890)—who experimented with curarine—the cardio-inhibitory fibres are paralysed more readily in the cat than in the dog, and more readily in the dog than in the rabbit. He found that in the rabbit 10 to 40 mgms. of curarine prevented stimulation of the splanchnic nerve from causing a rise of blood-pressure; this was 100–300 times as much as was required for momentary paralysis of the motor nerves. A still larger dose was required to prevent a rise of blood-pressure being caused by stimulation of the spinal cord. Dickinson and myself (1890) found in the rabbit that the pupillo-dilator nerve fibres of the cervical sympathetic were paralysed much more readily than its vaso-motor fibres, about 10 mgms. being required in one case and about 60 in the other. The drug used was purchased as curarine sulphate, but in subsequent experiments I have found that it was not much more active than the curari I have used in other experiments.

With the exception of Dickinson and myself—who attributed the effect of curari to an action on nerve cells—all observers who discussed the point of action of curari considered that it paralysed motor nerve endings in unstriated muscle and glands, just as it paralysed motor nerve endings in striated muscle.

The different pre-ganglionic nerve fibres investigated may be divided into classes according to the order of paralysis with increasing amounts of curari. The classification is based on ocular examination of the effect of stimulating the nerves 2 to 4 minutes after injecting curari. As I have said the amount required for paralysis varies in different cases. The amounts I give below are those which I estimate to be generally effective in a cat weighing 2 to 2·5 kilograms.

*Class I.* The inhibitory fibres of the vagus. These were paralysed before any other pre-ganglionic fibres I have investigated. It was beside my purpose to determine their ease of paralysis compared to that of skeletal nerves. I usually injected 1·5 to 2 c.c. of 1 p.c. curari as a first dose. This caused paralysis of the cardio-inhibitory fibres of varying duration. In one case in a cat of 2 kilo. body weight, I injected 1 c.c. Stimulation of the peripheral end of the peroneal nerve caused no movement; stimulation of the peripheral end of the vagus caused a fall of blood-pressure of about 10 mm. Hg. and a slowing of the heart from 40 to about 35 beats in 10 secs. (Fig. 1).

---

[1] Dilatation of the vessels of the ear is not necessarily absent in these circumstances.

*Class* 2. The secretory fibres of the chorda tympani, the secretory pupillo-dilator and pilo-motor fibres of the cervical sympathetic. These were paralysed for a short period by 5 c.c. of 1 p.c. curari. The pilo-motor fibres for the back and tail, and the sweat fibres of the feet belong, so far as my observations go, to this class.

*a* *b*

Fig. 1. Effect of 1 c.c. 1 p.c. curari upon the cardio-inhibitory nerve fibres of the cat. Stimulation of the peripheral end of the vagus (*a*) before, (*b*) after injection.

*Class* 3. The cutaneous vaso-constrictor fibres (cervical and lumbar sympathetic); the fibres of the cervical sympathetic for the nictitating membrane and eyelids. The amount of curari required for these varied more than that required for the nerve fibres in Class 2. They were generally but not always paralysed by 10 c.c. of 1 p.c. curari, and there was brief paralysis with 5 c.c.

As a rule I injected curari in two successive doses of 5 c.c. of a 1 p.c. solution, sometimes 10 c.c. initially. In Exp. 1 I give some details of the effect on the cervical sympathetic of successive injection of smaller amounts; since it brings out more clearly the progressive paralysis of the several nerve fibres, and will show also some other effects of curari which will be referred to in this and in subsequent papers.

EXP. 1. *Cat.* Chloroform; tracheotomy, A.C.E. Right cervical sympathetic tied and cut, causing flushing of right ear and other usual effects. Femoral vein for injection.

0 mins. Inject 3 c.c. 1 p.c. curari. Both ears flush, become equally red, some flushing of lips. Slight contraction of pupils during injection, dilatation afterwards. Right nictitating membrane less over cornea.

7 „ Stim. rt. c. sy.—all the usual effects promptly, but the pupil begins to contract in about 10 secs.

12 „ 3 c.c. 1 p.c. curari. After the end of the injection, both pupils dilate, begins first on right side. Slight flushing of ears and lips.

15 „ Stim. rt. c. sy.—prompt effect on the nictitating membrane, less on eyelids, slight on pupil and hairs of face; pallor of ear and lip.

20 „ 3 c.c. 1 p.c. curari. No visible effect.

23 „ Stim. rt. c. sy.—no effect on pupil; still distinct effect on nict. memb. and eyelids.

33 „ 3 c.c. 1 p.c. curari. No visible effect.

35 „ Stim. rt. c. sy.—Slight, but obvious, retraction of nict. memb., feeble movement of eyelids. No effect on pupil or hairs and doubtful effect on vessels of lips and ear.

In this experiment the preliminary contraction of the pupil following the first injection of curari, I take to be due to blocking of nerve impulses in the pupillo-dilator nerve cells of the superior cervical ganglion, and the subsequent dilatation to blocking of impulses in the ciliary ganglion. The second injection caused renewed dilatation of the pupil, but the third and fourth had no effect. The blocking of nerve impulses may then be taken as having been completed by the second injection. In the experiments in which two successive injections of 5 c.c. of 1 p.c. curari were made at a few minutes interval, the first always caused dilatation of the pupil, whether the superior cervical ganglion was excised or no, and the second had no effect on it. On this account, and on account of the effect of injecting other drugs after curari, I conclude that the preganglionic fibres of the sphincter of the iris are paralysed at about the same time as the pupillo-dilator fibres of the sympathetic.

### *Action of strychnine and brucine in increasing dose.*

Curari was first given in amount just sufficient to paralyse somatic nerves. The amount of strychnine hydrochlorate or brucine nitrate injected, varied but little in the several experiments. As a rule 5 mgms. of strychnine was first given then 50 mgms. then another 50 mgms.; in the case of brucine 5 mgms. was first given then 100 mgms. or 100 mgms. initially.

Since the animal was curarised the amount of strychnine which paralysed the cardio-inhibitory fibres could not be determined. In a recent experiment in which the brain and spinal cord were destroyed in A.C.E. anæsthesia, and curari not given, stimulation of the peripheral end of the vagus had no effect on the heart after injection of 20 mgms. of strychnine.

Fifty milligrams of strychnine caused temporary paralysis of the chorda tympani, of pupillo-dilator and secretory fibres of the cervical sympathetic and great decrease in the effect of stimulating its other fibres. The injection of another 50 mgms. completely paralysed for a time all the fibres of the cervical sympathetic. Brucine in a dose of 100 mgms. paralysed all the pre-ganglionic fibres investigated, viz. chorda tympani, cervical and lumbar sympathetic.

The order of paralysis of the several classes of nerve fibres was only investigated in a few cases, but in these it was practically the same as that found with curari. In the rabbit, Dickinson and myself (1890) found that curari, strychnine and brucine paralysed the pupillo-dilator fibres of the cervical sympathetic before the vaso-constrictor fibres; the difference in the time of paralysis being especially marked with brucine.

## THE STIMULATING EFFECTS OF NICOTINE AFTER CURARI, STRYCHNINE AND BRUCINE.

In the normal anæsthetised cat 0·1 to 0·2 mgm. of nicotine is sufficient to cause amongst other effects, dilatation of the pupil, retraction of the nictitating membrane, separation of eyelids, constriction of the arteries of the ear, and of the pads, erection of hairs, secretion of saliva, a large rise of blood-pressure, the rise of blood-pressure being sometimes preceded and sometimes broken by a fall owing to inhibition of the heart. The inhibition of the heart is however not constant.

Excess of anæsthetics, especially of chloroform, decreases the stimulation effects. In the experiments to determine whether curari prevents nicotine from stimulating nerve cells, I have usually injected 3 mgms. of nicotine, *i.e.* an amount much greater than sufficient to stimulate strongly the nerve cells when a paralysing drug is not given; it was usually injected about 3 mins. after the injection of curari, strychnine or brucine.

If a very large amount of curari is administered (about 10 c.c. of a 2 p.c. solution) the injection of a large amount of nicotine has no effect. In Exp. 2 a purified curari was used, *i.e.* a curari containing a greater percentage of curarine than the curari I ordinarily used. The injection of 18 mgms. of nicotine was without effect.

Exp. 2. *Cat.* Starch grains injected into one carotid artery peripherally and the brain above the corpora quadrigemina destroyed. Two injections of 100 mgms. of purified curari were given at an interval of 7 mins. The blood-pressure fell to 27 mm. Hg. Ten minutes after the second injection of curari, 3 mgms. of nicotine, and a minute later 15 mgms. had no effect on the blood-pressure, the eye, the hairs or the secretion of saliva; adrenaline caused a good rise of blood-pressure.

Obviously then a sufficient amount of curari paralyses all the peripheral nerve cells so far as the action of a considerable dose of nicotine is concerned.

When curari is given in two or three doses of 5 c.c. 1 p.c. curari, the effect of 3 mgms. of nicotine is confined or nearly confined to causing a rise of blood-pressure. In one case in which 10 c.c. of 1 p.c. curari was given in one injection and 3 mgms. nicotine given 5 mins. later, the rise was only 20 mm. Hg., in all other cases it was considerable. An example of this is given in Exp. 3. With the rise of blood-pressure there was in one case slight retraction of the nictitating membrane, and in one a doubtful trifling dilatation of the pupil.

Exp. 3. *Cat.* A.C.E. Left superior cervical ganglion excised. Right cerv. symp. tied and cut. Chordo-lingual n. tied and cut. Injection into femoral vein. Blood-pressure from femoral artery.

0 mins. 5 c.c. 1 p.c. curari. Bl.-pr. falls from 94 to 56 mm. Hg. Pupils dilate slowly to about ½ full (first on left side), slowly increase to ¾.

2 „ Stim. chordo-lingual, no secretion of saliva.

Stim. c. symp., no effect on pupil, other ocular effects slow.

7 „ 5 c.c. 1 p.c. curari. Bl.-pr. slowly rises from 46 to 48. No effect on pupil or eye.

9 „ Stim. c. symp., no effect.

11 „ Stim. c. symp., slow, slight effect on eye; on repeating, effect decreases and ceases.

17 „ 5 c.c. 1 p.c. curari. Bl.-pr. no effect; at 59.

Stim. c. symp., no effect.

19 „ 3 mgms. nicotine. Bl.-pr. rises from 62 to 145. No effect on pupil or eye.

24 „ 10 mgms. nicotine. Bl.-pr. rise 56 to 300. On right, is rather slow retraction of nict. memb. and separation of eyelids, with slight dilatation of the pupil. On left, very slight effect on nictitating membrane and eyelids, and trifling dilatation of pupil. Doubtful very slow erection of hairs on tail. No secretion of saliva.

Even with a fairly large amount of curari, the paralysis of the preganglionic nerves soon ceases to be complete, and 3 mgms. of nicotine given no long time after the curari produces several or all of its normal effects. In Exp. 4, an interval of 17 mins. was allowed between the two injections of 5 c.c. 1 p.c. curari; in this case there was, with 3 mgms. of nicotine, a marked effect on the nictitating membrane and eyelids in addition to a rise of blood-pressure. In Exp. 5 the nicotine was not injected until 23 mins. after the curari, and all the normal effects except inhibition of the heart were produced.

Exp. 4. *Cat.* A.C.E.

0 mins. 5 c.c. 1 p.c. curari.
17 „ 5 c.c. 1 p.c. curari.
20 „ 3 mgms. of nicotine caused besides a rise of blood-pressure distinct retraction of the nict. memb. and separation of the eyelids, but had no effect on the hairs of the tail nor on the salivary secretion. (The pupil was not observed.)

Exp. 5. *Cat.* A.C.E.

0 mins. 5 c.c. 1 p.c. curari.
6 „ 5 c.c. 1 p.c. curari.
29 „ 5 mgms. nicotine caused a rise of blood-pressure, dilatation of pupil and other ocular effects, erection of hairs on the tail, and slight secretion of saliva.

On the other hand protracted low blood-pressure reduces the excitability of the nerve cells, so that if the injection of nicotine is long delayed and the blood-pressure remains low, the effect of nicotine is apt to be lessened as in the following experiment.

Exp. 6. *Cat.* A.C.E. and urethane. The 2nd and 3rd lumbar nerve roots tied and cut. Stimulation of the 2nd lumbar roots caused erection of hairs in the region of the 3rd lumbar nerve, downwards to about ⅓ of the tail, copious secretion on the pads, and marked pallor. Stimulation of the 3rd lumbar roots caused erection of hairs in the region of the 4th lumbar nerve downwards to the end of the tail, slight secretion on the pads and fair pallor.

Inject 10 c.c. 1 p.c. curari.

Stim. nerve roots—no effect.

Stim. nerves peripheral of spinal ganglion, *i.e.* the grey rami—strong erection of hairs from each nerve in its own skin segment but not elsewhere.

Leave for half-an-hour.

Stim. nerve roots—trace of effect on hairs and on tint of foot, no secretion.

Inject 5 mgms. nicotine—no effect on hairs, feet, or eye.

Inject 30 mgms. nicotine about 7 mins. later—strong erection of hairs; nict. memb. retracted and eye opened, few small drops of sweat on pads.

In previous experiments on the sacral vesico-motor fibres (1911) I have shown that the stimulating effect of nicotine after curari depends upon the relative concentration of nicotine and curarine. The experiments given above afford evidence that the relative concentration governs the stimulating effect in all cases. But the effect of injecting a large dose of nicotine a few minutes after 3 mgms. was not always that which occurred in the experiments I have given. Sometimes the second larger dose had no effect other than that caused by the first dose, *i.e.* a rise of blood-pressure. The absence of additional effect, I take it, means that in these cases the paralysis produced by curari was more profound. At the same time the possibility that after a certain large amount of curari, no stimulation is possible by nicotine, is not definitely excluded.

It may be recalled that the degree of stimulating effect depends in part on the duration of stimulation, and that in such effects as a rise of blood-pressure which take time, a large dose of nicotine injected quickly may produce less effect than a small one on account of the rapid paralysis. On the other hand, when nicotine displaces curari, a certain rapidity of displacement is necessary for effective stimulation. Since nicotine produces its effects in part by stimulation of the central nervous system—especially of the bulbar centres—the degree of effect produced by it depends also upon the excitability of the central nervous system, and, if the nerves are intact, upon the degree of peripheral block to any impulses which are set up.

Since it was impossible to determine the exact degree of paralysis produced by curari, and since the first injection of nicotine tends to reduce the irritability of nerve cells to subsequent injection, I adopted another method to show that a high concentration of nicotine overcomes the curari paralysis. Curari was injected until stimulation of the cervical sympathetic had no effect. Then, immediately, 1 p.c. nicotine was applied locally to the superior cervical ganglion, it caused maximal dilatation of the pupil and the other effects normally caused by stimulating the cervical sympathetic (cp. Exp. 7).

Exp. 7. *Cat.* A.C.E. Tie and cut both cervical sympathetics, and expose both superior cervical ganglia.

0 mins. 5 c.c. 1 p.c. curari.
2 „ Stim. right c. sy.—gives all effects.
4 „ 5 c.c. 1 p.c. curari.
7 „ Stim. right c. sy.—trace of retraction of nict. memb., no other effect.
Stim. left c. sy.—trace of retraction of nict. memb., no other effect.
8 „ Brush warm 1 p.c. nicotine on right sup. cerv. gang., strong effect on nict. memb., eyelids, and pupil; slight erection of hairs.
12 „ Stim. left c. sy.—eye opens.
14 „ 5 c.c. 1 p.c. curari.
18 „ Stim. left c. sy.—trace of retraction of nict. memb., no other effect.
18½ „ Brush warm 1 p.c. nicotine on left sup. cerv. gang., strong effects on nict. memb., eyelids, pupil, and hairs.

In a recent experiment on a decerebrated animal a good example was obtained of the difference in effect of a small and of a large dose of nicotine on the blood-pressure after curari had been given (Fig. 2). On account of the decerebration, the effect on the eyes could not be observed. It will be seen (Fig. 2) that 2 mgms. of nicotine caused only a trifling slow rise of blood-pressure, whilst 25 mgms. injected 2½ minutes later caused a quick large rise.

From the results given above it is I think clear that the excitation of nerve cells by nicotine, after curari has been administered, depends—at any rate within wide limits—upon the relative concentration of nicotine and curarine in contact with them.

The effects produced by the second injection of nicotine given at various times after the first were broadly speaking such as would be expected on the theory that nicotine antagonises the paralysing action of curari on the nerve cells in inverse proportion to the ease of paralysis by curari as determined by nerve stimulation. But there were exceptions to this. The most notable was the occurrence of a considerable rise of blood-pressure with little or no effect on the nictitating membrane. There was also at times distinct, though not great, dilatation of the pupil without erection of hairs or secretion of saliva; it is true that the relative time of paralysis of the nerve fibres causing these effects were not accurately determined, but the experiments on nerve stimulation did not suggest that the time was markedly different. In considering the results produced by nicotine after a drug which paralyses nerve cells we have to consider how far they are produced by stimulation of the nerve cells and how far by stimulating the nerve fibres which cause secretion of adrenaline. It was shown by Cannon, Aub, and Binger (1912) that nicotine liberates adrenaline. It was noticed by Dickinson and myself (1890) that in the cat, nicotine caused large dilatation of the pupil and other ocular effects after excision of the superior cervical ganglion. Dale and Laidlaw (1912) found that these effects in cats anæsthetised by A.C.E. or ether were not produced by nicotine if the suprarenal glands as well as the superior cervical ganglion were excised, and they concluded that with these anæsthetics, dilatation of the pupil produced by nicotine after excision of the superior cervical ganglion showed a liberation of adrenaline.

Fig. 2. Cat, decerebrated. 4 c.c. of 2 p.c. purified curari had been injected before the tracing shown in the figure. The 25 mgms. of nicotine were injected 2½ mins. after the 2 mgms.

In my earlier experiments, as I have said, A.C.E. mixture (after chloroform) was the sole or the chief anæsthetic. In several of the experiments, the superior cervical ganglion was excised on one side, and the larger dose of nicotine given after the first 3 mgms. caused more or less dilatation on the excised side. Examples are given in Exp. 3 in which the dilatation was trivial and in Exp. 8 in which it was greater. On the basis of these facts it follows that curari paralyses the adrenaline secreting fibres, and that this paralysis can be overcome by nicotine.

There is however one further complication. Dale and Laidlaw (1912) observed that when urethane was employed as an anæsthetic, 1–3 mgms. of nicotine caused dilatation of the pupil and protrusion of the nictitating membrane after excision both of the superior cervical ganglion and of the suprarenals. They left it an open question whether the effects were produced by peripheral stimulation or by liberation of a substance from cells other than the chromaffin cells. In these observations, Dale and Laidlaw have I think overlooked the effect of nicotine upon the ocular nerves. Paralysis of the ciliary ganglion by nicotine necessarily causes dilatation of the pupil if the tectal centre is exercising any tonic action on the sphincter of the pupil. In urethane anæsthesia the pupil is small and the tone presumably strong. It was shown by Anderson and myself (1892) that stimulation of the 6th nerve causes protrusion of the nictitating membrane. Nicotine stimulates the 6th nerve before paralysing it. The effects of nicotine on the eye after removal of the superior cervical ganglion and of the suprarenals in animals anæsthetised by urethane are then adequately accounted for by its action on the ocular nerves. I may mention that after excision of the superior cervical ganglion I have seen primary contraction of the pupil on injecting a small amount of nicotine (1 mgm.); this might be due either to central stimulation or to stimulation of the ciliary ganglion before the paralysis sets in. In my experiments on the action of nicotine after curari, the ciliary ganglion and ocular nerves were paralysed by curari before nicotine was given.

A part of Dale and Laidlaw's account seems to imply some additional action of nicotine, but the details given are hardly sufficient to allow a decision to be made. (*a*) They observed that when the brain and spinal cord were destroyed, the sup. cerv. gang. and the suprarenals excised, nicotine produced the same effects as in urethane anæsthesia, but it is not mentioned that the pupils were large; if they were small there would probably be some sphincter tone from incomplete destruction of the lower centres. (*b*) On the theory I have given above, whilst nicotine would not produce an effect in A.C.E. anæsthesia, when the pupil is large, it would produce it in the later stage when the pupil is small. (*c*) They found that nicotine produced the above mentioned effect when it caused no rise of blood-pressure. I tried one experiment on this point. In urethane anæsthesia, the left superior cervical ganglion and the suprarenals were excised. 10 mgms. of nicotine were injected. Four minutes later another 10 mgms. were injected; this caused a slight slow rise of blood-pressure, but had no effect on the left eye.

As mentioned above **Dale** and **Laidlaw** suggest that nicotine may perhaps liberate a pupillo-dilator substance from some other gland than adrenaline. In view of the almost universal stimulation of nerve cells by nicotine, it is I think practically certain that all glands of internal secretion which are governed by nerves are stimulated to secretion by nicotine, and the possibility that the effects of nicotine may be partly due to liberation of other hormones than adrenaline, though there is at present no evidence for it must be borne in mind.

The effects produced by the liberated adrenaline will depend upon the relative sensitiveness to it of the several peripheral tissues, and when nicotine is given some time after the curari, the relative sensitiveness both of nerve cells and of peripheral tissues may be altered by the prolonged low blood-pressure. We may, I think, confidently refer to these causes the smaller divergencies spoken of above between the results produced by a large dose of nicotine after curari, and those to be expected from the order of paralysis of nerve cells by curari. Individual variations will obviously be caused by variations in the amount of adrenaline stored in the glands at the time of injecting nicotine.

In one case with curari and in one case with brucine the dilatation of the pupil on injecting 3 mgms. of nicotine (cp. Exp. 8, 2nd injection) was practically the same on the side with excised and on that with intact ganglion, and there was no effect on the nictitating membrane. I infer that the nicotine did not stimulate the nerve cells connected with either of these structures, and that it caused the ocular effects by liberating adrenaline. The facts make it probable that the adrenaline secreting fibres are paralysed by curari and brucine not only later than the pre-ganglionic pupillo-dilator fibres, but also later than the pre-ganglionic fibres for the nictitating membrane.

**Elliott** (1913) considers that there are no peripheral nerve cells on the course of the nerve fibres causing secretion of adrenaline, mainly because he finds that section of the splanchnic nerve in the cat causes degeneration of a number of myelinated fibres in the suprarenal gland, and that nerve cells in the medulla are rare in this animal. On the other hand both **Dogiel** (1894) and **Renner** (1914) describe nerve cells in the medulla giving off nerve fibres which end in the chromaffin cells. Thus the question whether nicotine stimulates and paralyses nerve cells on the course of the secretory fibres, or the chromaffin cells direct, requires further investigation.

Liberation of adrenaline does not however seem to me to account satisfactorily for the occurrence of a considerable rise of blood-pressure with no effect on the eye, and this, as I have said, commonly occurs when 3 mgms. of nicotine are given after two injections of 5 c.c. of 1 p.c. curari. In the conditions of the experiments, *i.e.* with a more or less dilated pupil, a rise of blood-pressure is no doubt a more delicate reaction for adrenaline than pupil dilatation, but on injecting adrenaline I have not found a considerable rise of blood-pressure without pupil dilatation. I have made one experiment in which the brain above the posterior corpus quadrigeminum was destroyed and the suprarenals tied off from the circulation. As the body weight was 3·8 kilos, three injections of 5 c.c. 1 p.c. curari were made at intervals of 7–8 mins. Stimulation of the cervical sympathetic still caused slight retraction of the nictitating membrane but there was no other effect. The vagus had no effect on the blood-pressure. The injection of 3 mgms. of nicotine caused a rise of blood-pressure from 60 to 166 mm. Hg. (Fig. 3) with only slight retraction of the nictitating membrane and a doubtful dilatation of the pupil. The result I think shows that the vaso-constrictor nerve cells were much more stimulated than other nerve cells. Now so far as simple inspection goes, I have not found the cutaneous vaso-constrictor nerve cells either in the cervical or in the lumbar sympathetic to be paralysed later by curari than the nerve cells on the course of the fibres to the nictitating membrane. It is true that in the cat complete absence of vaso-constriction is difficult to determine by simple inspection and that a slight contraction in all the vessels of the body might have a not inconsiderable effect on the blood-pressure. But the results do not seem to me to be adequately explained in this way, and pending further experiments, I conclude that the vaso-constrictor nerve cells of the abdominal viscera are paralysed by curari somewhat later than the

Fig. 3. Rise of blood-pressure caused by 3 mgms. of nicotine after a large dose of curari in a decerebrate cat in which the suprarenals had been tied off from the circulation. (The second slight rise may have been due to the injection of 2 c.c. of Ringer's fluid made for the purpose of forcing on any nicotine remaining in the vein.)

cutaneous vaso-constrictor nerve cells. The visceral vaso-constrictor nerve fibres and the adrenaline secretory nerve fibres, on this view, form a Class 4 to be added to the Classes given on p. 252. The question of the difference in the time of paralysis of cutaneous and visceral nerve cells I am investigating more fully in relation to the action of strychnine to be dealt with in a subsequent paper.

The injection of curari, when the vagi were cut, rarely had any marked effect upon the rate of heart beat; the usual form of curve caused by the first injection of 5 c.c. 1 p.c. curari is shown in Fig. 4; the extent of the fall varies with the rate of injection and other conditions. In such cases 3 mgms. of nicotine in causing a rise of blood-pressure had little or no effect on the heart beat (Fig. 1). In one case only—that of Exp. 3—curari caused a great slowing of the rate of beat, which increased with each injection. The slowing was probably due to the diminished circulation acting upon the heart enfeebled by the large excess of A.C.E. which was administered. The injection of 3 mgms. of nicotine caused great quickening of the beat (cp. Fig. 5). Whilst I think it probable that the accelerator nerve cells are paralysed with those of Class 2, the relative time of paralysis can only be determined by observations in which the upper thoracic nerves are stimulated, for if the heart beat is fast, stimulation of the accelerator nerves may be unable to increase it,

Fig. 4. *Cat*, decerebrated. Fall of blood-pressure caused by 5 c.c. 1 p.c. curari. The variations of pressure during the injection were caused by slight movements.

and if it is slow, a quickening may be caused either by increased circulation of blood through it, or by liberation of adrenaline.

The time of paralysis of the bulbar and sacral vaso-dilator fibres is difficult to determine with any exactness because of the low blood-pressure caused by curari, but so far as my observations go, the vaso-dilator nerve cells belong to Class 2 rather than to Class 3, though there was some indication of their being paralysed somewhat later than the pupillo-dilator and secretory fibres.

Fig. 5. *Cat.* A.C.E. in excess. To show slowing in rate of heart beat very rarely caused by curari and the rise of blood-pressure with increase of rate of heart beat caused by subsequent injection of 3 mgms. of nicotine.

### *Effect of nicotine after strychnine and brucine.*

The antagonism by nicotine of the paralysis of nerve cells by strychnine and brucine is the same in its general features as the antagonism of the paralysis by curari, but the paralysis produced by 100 mgms. of strychnine appeared to be of less duration, and that produced by 100 mgms. of brucine appeared to be of longer duration, than that caused by 10 c.c. of 1 p.c. curari. An example of an experiment with brucine is given in Exp. 8. In this, the second dose of 3 mgms. of nicotine given after an interval of 40 mins. had no effect on the hairs, on secretion or on the nictitating membrane, and in another experiment 10 mgms. of nicotine injected slowly 12 mins. after 100 mgms. of brucine caused a rise of blood-pressure of only 22 mm. Hg. and had no other effect.

Exp. 8. *Cat.* A.C.E. and urethane anæsthesia. Left superior cervical ganglion excised. Right cervical sympathetic cut. Injection in femoral vein. Blood-pressure taken in femoral artery. 1·8 c.c. 1 p.c. curari.

3 mgms. of nicotine injected 7 mins. after 100 mgms. brucine nitrate caused a very slow rise of blood-pressure from 64 to 92 mm. Hg. without other effect.

3 mgms. of nicotine injected 40 mins. later caused a rise of blood-pressure from 58 to 138 mm. of Hg., the only other effect observable was a trace of dilation of both pupils.

20 mgms. of nicotine injected 18 mins. later caused a rise of blood-pressure from 59 to 139 mm. Hg., fairly good erection of hairs on the tail, complete but slow retraction of the nictitating membrane and separation of the eyelids on the right side (cervical symp. cut) but no movement of those on the left side (ganglion excised); both pupils slowly dilated after an interval; and apparently slightly earlier on the right side. There was no secretion of saliva.

3 mgms. of pilocarpine cause primary fall of blood-pressure, followed by a rise, copious secretion of saliva, and slow constriction of the pupil.

My previous experiments on the nerve cells on the course of the sacral nerves to the bladder (1910 and 1911) show that these must be put in a different class from any of those dealt with in this paper. In the stage of curari action in which other nerve cells are nearly or completely paralysed, stimulation of the sacral pre-ganglionic fibres of the bladder causes great after-contraction, and if the stimulation be renewed during the after-contraction, it causes inhibition. A similar change in the effect of stimulation is produced by nicotine, by strychnine, and probably by all drugs having a paralysing action on peripheral nerve cells. The occurrence of this 'rebound and reversal' has been shown as a stage in the paralysis of some other oro-anal nerve fibres. It was first demonstrated in the case of the action of the vagus on the heart by Dale, Laidlaw and Symonds (1909 and 1910). Part of the reaction of the vagus to curari was earlier described by Wundt (1860) and by Boehm (1875). A similar change was shown in the action of the chorda tympani on the sub-maxillary gland by myself (1911) and independently and more fully by Dale and Laidlaw (1911), but as the investigations stand at present the stage of rebound and reversal rapidly passes into complete paralysis with increasing amount of the paralysing drug except in the case of the sacral vesico-motor fibres.

I may add some notes on the state of the circulation after more or less considerable paralysis of peripheral nerve cells by curari. The primary effect is flushing both of the skin and of the intestine. Later the skin becomes pale—though the small arteries and veins are obvious—whilst the abdominal viscera, especially the intestine, remain flushed. If the heart beat becomes weak, the veins enlarge. Vigorous artificial respiration is required to keep the arterial blood scarlet. The blood-pressure can be raised temporarily by many means, but none that I have tried will do so to any important extent for more than 7 to 10 minutes. In my earlier experiments, gelatine—which I tried in consequence of Barcroft and Knowlton's experiments—was more effective than Ringer's fluid, but less effective than ·04 p.c. barium chloride in Ringer's fluid. Gelatine tended to cause agglutination of red corpuscles; if the mesenteric veins were compressed the corpuscles at once ran together in clumps leaving clear plasma between them, but the lumps at once broke up on releasing the pressure. The effect of barium chloride is partly due to intestinal contraction, it usually caused defæcation. I have since tried 6 p.c. gum with sodium carbonate recommended by Bayliss and which has been found useful in 'wound shock'; this also causes only a temporary rise. Pituitrin (1 c.c. of Burroughs and Wellcome's infundibular extract) causes some rise of blood-pressure but it does not last long. Adrenaline is invaluable in these experiments, for it revives the heart beat when other means are useless, and by injecting a small amount every three or four minutes a better circulation is obtained than in any other way.

## Summary and Conclusions.

1. Additional instances are given in which curari, strychnine and brucine paralyse pre-ganglionic nerves in the cat, and I conclude that these poisons have a more or less paralysing action on all pre-ganglionic nerves.

2. The following is the order of ease of paralysis as determined in most cases by nerve stimulation, and in some cases by injecting nicotine after the paralysing drug.

(*a*) Cardio-inhibitory fibres.

(*b*) Secretory fibres of the chorda tympani, sweat fibres, pilo-motor, pupillo-dilator and pupillo-constrictor fibres. Probably also bulbar and sacral vaso-dilators.

(*c*) Fibres for the nictitating membrane and eyelids and, so far as simple inspection goes, cutaneous vaso-constrictor fibres.

(*d*) Probably adrenaline secreting fibres and abdominal vaso-constrictors.

A difference in the time of paralysis of abdominal and cutaneous vaso-constrictor fibres would allow reflex vascular constriction in the skin to be distinguished from that in the abdominal viscera, provided the central mechanism is not the first to be paralysed.

3. Nicotine if given in sufficient amount antagonises the paralysing action of curari, strychnine and brucine and produces its usual stimulating effects. The order of ease of stimulation, other things being equal, is in the several cases inversely proportional to the ease of paralysis.

4. Since nicotine stimulates by an action on the peripheral nerve cells and since curari, strychnine and brucine do not paralyse post-ganglionic nerves, the paralysis produced by curari, strychnine and

brucine is produced by an action on the peripheral nerve cells. If, as Elliott holds, there are no nerve cells on the course of the adrenaline secreting fibres, the paralysing action must in this case be direct on the chromaffin cells.

5. In the action of nicotine and physostigmine on frog's muscle, I have distinguished (1914) between the stimulus which produces a slow local tonic contraction and that which produces the quick conducted contraction or twitch. In the case of the tonic contraction whether there is stimulation or no after curari has been given depends on the relative concentration of the paralysing and stimulating alkaloid. In the case of the twitchings, whether there is stimulation or no depends, so far as the observations go, upon the absolute concentration of the paralysing alkaloid. The antagonism by nicotine of the action of curari, strychnine and brucine on nerve cells is of the former kind—at any rate between wide limits of concentration.

6. No means have been found of permanently raising the blood-pressure to any important extent after a large dose of a poison paralysing peripheral nerve cells.

## REFERENCES.

1856. Cl. Bernard. Leçons s. l. effets d. substances toxiques (June 1856), p. 348. Paris, 1857.
1856. Kölliker. Virchow's Arch. 10. 3.
1860. Wundt. Quoted from Boehm.
1863. v. Bezold. Unters. ü. d. Innerv. d. Herzens, p. 312. Leipzig.
1873. Eckhard. Beiträge, 7. 67.
1875. Boehm. Arch. f. exp. Path. u. Pharm. 4. 351.
1877. v. Frey. Ludwig's Arbeiten, p. 98.
1889. Feotistoff. Bull. d. l'Acad. Imp. d. Sci. d. St Petérsbourg, 13.
1890. Tillie. Arch. f. exp. Path. u. Pharm. 27. 1.
1890. Langley. This Journ. 11. 123.
1890. Langley and Dickinson. Ibid. 11. 509.
1892. Langley and Anderson. Ibid. 13. 460.
1894. Dogiel. Arch. f. Anat. (u. Physiol.), p. 90.
1895. Langley and Anderson. This Journ. 19. 131.
1901. Langley. Ibid. 27. 224.
1909. Dale, Laidlaw and Symonds. Proc. Physiol. Soc. p. xiii. (This Journ. 39.)
1910. Langley. Ibid. p. xlii. (This Journ. 40.)
1910. Dale, Laidlaw and Symonds. This Journ. 41. 1.
1911. Langley. Ibid. 43. 125.
1911. Dale and Laidlaw. This Journ. 43. 196.
1912. Cannon, Aub, and Binger. Journ. Pharm. and Exp. Therap. 3. 379.
1912. Dale and Laidlaw. This Journ. 45. 1.
1913. Elliott. Ibid. 46. 285.
1914. Langley. Ibid. 48. 107.
1914. Renner. Deutsch. Arch. f. klin. Med. 114. 473.
1915. Camis. Rev. d. l. Facultad d. Agron. 11. 29.

# THE CONTROL OF THE PHYSICAL REGULATION OF THE BODY TEMPERATURE. BY J. M. O'CONNOR.

(*From the Physiological Laboratory, University College, Dublin.*)

THE regulation of the body temperature exhibits, as is well known, two aspects of different nature and prevailing, in the main, under opposed conditions. Taking their nomenclature from Rubner these are known as the chemical regulation, exhibited in the cold, and the physical regulation, paramount at high external temperatures. In a previous paper (1) I was able to show that chemical regulation was under a dual control. Its onset depends on the brain temperature being below a certain point, its intensity on the extent to which the skin temperature is below this point. The investigation embodied in the present paper is directed to an analysis of the factors governing the physical side of the regulation.

There are three methods by which an animal can regulate the removal of heat from its body: the cutaneous blood vessels, the secretion of sweat and the respiration. The vasomotor mechanism is certainly the most essential of these and the present paper is occupied with it alone. Its essential importance was not however the only justification for this limited consideration. The fact that the vigour of respiratory movement is concerned with temperature regulation only in a very subsidiary fashion makes this but little suited and technically difficult as a method of reaching any general principles, and experience on the secretion of sweat did not encourage hope of detecting any proportionality such as might be looked for between the vasodilation and the governing temperatures of the body.

The general plan of enquiry adopted consisted in heating anæsthetised animals by various means and following at the same time the blood flow through the leg, endeavouring to classify the changes observed and seek common causes for them in the temperatures of various portions of the body.

The technic may be considered in four parts—the anæsthetic, the methods of warming the animal, the measurement of the temperature and the determination of the blood flow through the leg.

With the exception of one on a dog (Exp. 11, Table II), all the experiments considered were done on cats. In the earlier experiments intraperitoneal injection of alcoholic solution of chloretone as recommended by **Rowe**(2) was used. This was found to give a long and uniform anæsthesia but the dosage was unreliable. The following procedure gave the best results. The animal received 1 c.c. of a 40 per cent. solution and then ¼ c.c. doses every ten minutes until the anæsthesia was sufficiently deep. Later veronal in 0·6 grams pro kilo per os was used, with occasionally the addition of chloretone to deepen the veronal sleep. The blood-pressures under veronal narcosis were frequently remarkably high. The final experiments were done with urethane narcosis (1½ grams pro kilo per os) which I found to be the most satisfactory.

A variety of methods of heating the animal were adopted to meet various requirements. In most of the experiments the animal was warmed by heating the copper warmer of the operating table on which it was lying. This crude method had advantages: the animal was accessible to adjustments such as the removal of clots from the blood-pressure cannula; further owing to the uneven heating the temperatures of different parts of the body were more widely separate than with any uniform heating arrangement, and it was consequently easier to pick out the common and presumably causal point of temperature in a series of experiments showing vasodilation on warming. Uniform heating was generally done by suspending the animal with a wire hammock in a bottle-shaped metal vessel open at both ends. The wall of the vessel was heated by an electric current through an iron wire wound round an asbestos covering. The leg projected through a perforation in a cushion covering the larger end of the vessel into the apparatus for measuring the blood flow, while the narrow end admitted sufficient ventilation. Heating the head alone was accomplished by a current of hot water (circa 60°) flowing through a tube under the carotids. Cooling the head was done in a similar fashion. Where more rapid heating was required the head of the tracheotomised animal was packed in a box which was heated electrically. Attempts made to heat the brain directly by an electrical heater or double current tube plunged into the brain failed.

Temperatures were measured by a galvanometer and copper eureka couples playing against a couple in a bath of known temperature. As a rule the temperatures of the brain, rectum and subcutaneous tissues on both sides of the body were taken. The brain thermocouple was fixed through a cork inserted into a trephine hole over the cerebrum. The rectal temperature proving of no demonstrable significance was not taken in the later experiments. The temperature of the subcutaneous tissue—or skin as it may be called for convenience—proved of such importance that reliance on two points only seemed insufficient and consequently in some of the later experiments the two separate skin records were replaced by eleven couples in series with the standard couple and distributed as diffusely as possible over the body surface. With uniform heating this gives a more correct average than a small number of separate couples. As however the insertion of eleven proved rather severe and the chances of one or more coming free and spoiling the experiment were too great the later experiments were done with five couples placed two on each side and one on the back of the head. All temperatures are centigrade.

For measuring the blood flow the usual plethysmographic method was considered unsuitable and instead the quantity of heat given off by the leg was measured. Apart from the fact that the plethysmographic curve indicates only the rate of change of the blood flow, experience pointed to its inapplicability. The usual air-plethysmographic curve in a long experiment would seem to be at least as much a record of changing temperature as of changing volume. The method used for measuring the heat given off by the limb was based on Hill's(3) calorimeter for small animals. It went through changes, but most of the experiments were done with the following arrangement. The chamber for the foot consisted of a piece of "red fibrous tubing" ¾ cm. thick and 4½ cm. bore diameter. This was lined by a coil of narrow bore copper tubing, the two ends of which passed out through the distal end of the fibrous tube which was sealed up with gutta percha. The ends of the copper tubing were connected with two small metal chambers containing the terminals of a thermopile (thirteen copper eureka couples) connected up by a key with the galvanometer. The chambers were connected up respectively with a water supply at a constant height and an outflow tube. The water flowing out was collected and measured periodically. Within the tube for the foot there also lay a loop of resistance wire (manganin) enclosed in glass tubing. The ends of this were connected to a source of current with resistance box and means of measuring the current flow and voltage at the ends of the heating wire. This arrangement copied from Macdonald(4) served to reduce the otherwise confusing latency of the instrument. The foot tube and thermopile chambers lay within a wooden box through a hole in which the open end of tube projected to permit insertion of the foot. The box was filled up with sawdust.

At the beginning of the experiment the foot was attached to a piece of wire which passed from the outside through the box and a hole in the closed end of the foot tube. The foot was then drawn in as far as the heel, the free end of the wire fastened to the table, and the heel packed around with cotton wool. A sufficient amount of current was sent through the heating circuit to bring the galvanometer reading from the thermopiles to a definite point, and throughout the experiment the reading was kept as far as possible at this point by varying the current. This of course could not be always achieved immediately as is shown by the oscillating appearance of some records (for example Fig. 1). The readings of the various body temperatures and of current flowing through the galvanometer were taken every five minutes. At the end of the experiment when the animal had been killed the amount of current necessary to keep the galvanometer reading at the standard point of the scale was determined. The product of the volt and ammeter readings at any time subtracted from the product required when the circulation had ceased gave a measure of the heat given off by the blood flowing through the leg at that time.

At this point we may consider some objections to the apparatus as a means of measuring the blood flow. The apparatus was not sufficiently well insulated to recover all the heat given out. Controls showed that about one-third of the heat was lost but as comparative values only were required this was not a serious objection. The arrangement contains the further assumption that all the heat given off by the leg was heat conveyed to it by the blood. As the cat's leg contains little but skin, bone and tendon there is no grave error involved in this. As the animal is heated up the blood reaching the leg also naturally becomes warmer and consequently different parts of the experiment are without correction not strictly comparable. The error introduced is however so relatively insignificant that the correction was not attempted. An increase in the blood supply causes not only more heat to be given off but would also, in spite of the arrangement for keeping the apparatus at a constant temperature, probably cause a consumption of heat in warming the leg itself. This would be an objection to any attempt at finer deductions from the experiment. As however such finer deductions proved to be devoid of prospect this difficulty does impair the value of the material for this paper. A similar case is furnished by the assumption necessarily made that the temperature of the blood leaving the leg was always the same. As the experiments were directed to the study of vaso-dilatory and vaso-constrictive phenomena allowance should be made for varying blood-pressure. The blood-pressure was taken in a large number of experiments and found to vary very considerably in the course of an experiment as can be seen from Fig. 5. In none of these however had the allowance for the blood-pressure sufficient influence to change the character of the record from vasomotor standpoint and consequently in some experiments in which the technical complication of a blood-pressure record endangered the prospects of success the attempt to obtain it was not made.

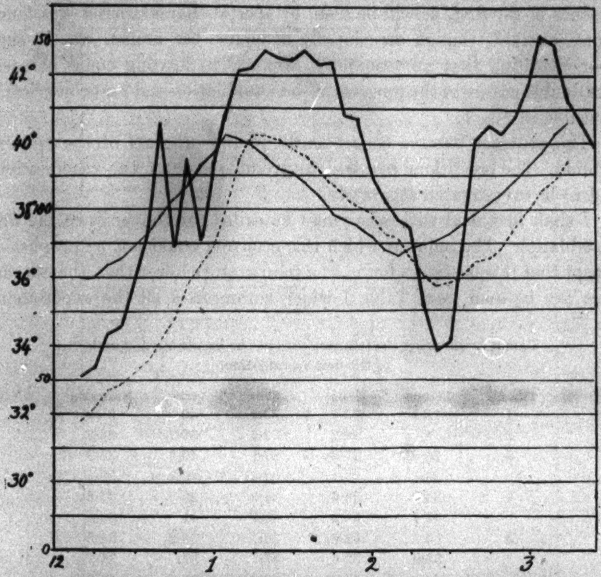


Fig. 1 (Exp. 12, Table I and Exp. 3, Table IV). Abscissa—time of observation. Thick line—heat loss from foot (watts × 100 uncorr.). Thin line—brain temperature. Broken line—skin temperature (five couples in series). Heating on from beginning of exp. to 1.5 and from 2.10 to end.

The point at which a vasodilation commences is usually well marked but occasionally admits of doubt. Dilation is always taken as having started when two successive points show a reading above the preceding value.

If an animal be anæsthetised and the experiment fitted up in the manner described its temperature always falls below the normal approaching steadiness at about 32° C. On heating such an animal, in a number of experiments two definite increases in the heat given off might be noticed, one occurring at a comparatively low point, the second at a much higher temperature. The first of these could be reckoned on in an animal in good condition. The second distinguishable even in the absence of the first, as will be seen, by special characteristics was much more constant, though occasionally of course the animal was in such poor condition that no vasomotor response to heating could be seen. The double nature of the progress of the vasodilation and vasoconstriction is visible in Fig. 1.

The problem became consequently in the progress of the enquiry twofold. The conditions required to produce each of the vasodilations had to be investigated separately.

I shall first deal with what may be called for convenience the first vasodilation. The point at which this occurred could not be prophesied except that it was always found at a temperature lower than the normal. This can be seen from Table I which summarises all the experiments

TABLE I. Showing the temperatures at the beginning and end (*e*) of the first vasodilation.

| Exp. no. | Rectum | Brain | Skin | Skin | Mean skin |
|---|---|---|---|---|---|
| 1 | 35·1 | 35·1 | 33·1 | 40 | 36·6 |
| 2 | — | 34·2 | 33·9 | 39·7 | 36·7 |
| 3 | 36·8 | 37·8 | 37·1 | 40·3 | 38·7 |
| *e* | 36·5 | 38·1 | 37·4 | 40·8 | 39·1 |
| 4 | 36 | 35·8 | 34·9 | 39·6 | 37·3 |
| *e* | 37·7 | 36·2 | 35·9 | 41 | 38·4 |
| 5 | 33·3 | 32·6 | 32·1 | 36·2 | 34·5 |
| *e* | 33·9 | 32·8 | 32·7 | 37 | 35·9 |
|  | 33 | 32·9 | 32·6 | 35·9 | 34·2 |
| 6 | 36·4 | 34·5 | 33·3 | 40·4 | 38·4 |
| 7 | 33·6 | 33·4 | 33·5 | 35·3 | 34·4 |
| 8 | — | 35·6 | 34·7 | 40·1 | 37·4 |
| 9 | — | 34·6 | — | — | 34·7 |
| 10 | — | 36·1 | — | — | 36·5 |
| *e* | — | 36·5 | — | — | 32·0 |
| 11 | — | 36·1 | — | — | 35·2 |
| 12 | — | 36·6 (?) | — | — | 32·9 |
| *e* | — | 36·9 | — | — | 36·6 |
|  | — | 37·3 | — | — | 35·9 |
| 13 | — | 36·3 | — | — | 33·3 |
|  | 35·2 | 35·4 | 34·4 | 38·9 | 35·9 |
|  | ±·8 | ±·5 | ±·9 | ±1·2 | ±1·1 |

showing this vasodilation. It became consequently necessary to determine in separate experiments whether it was produced by the brain temperature rising above a certain point or through some change in the skin temperature. The solution of the problem gave no difficulty. The vasodilation could be produced by heating the blood going to the head. A vasodilation so produced could be stopped without interference with the general skin temperature by cooling the carotid blood. An experiment of this type is shown in Fig. 2. In this, after preparation had been made for heating the carotid blood while, owing to the temperature of the table, all temperatures were rising, a vasodilation set in. The carotids were cooled instead and the vasodilation ceased sharply, but was again produced by warming the carotids. As can be seen all three changes

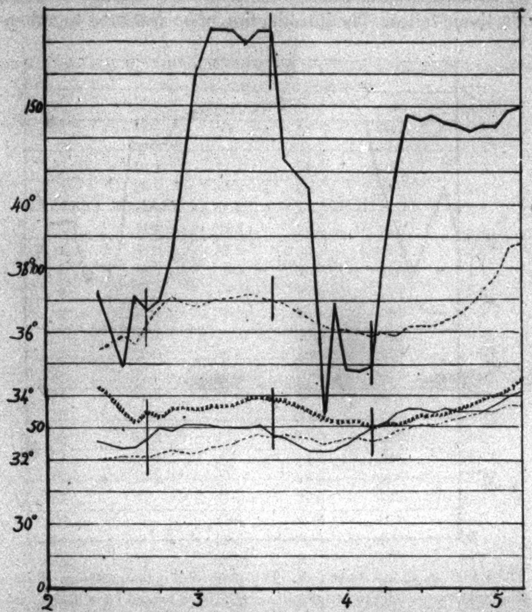


Fig. 2 (Exp. 5, Table I). Thick line—heat loss from foot. Thin line—brain temperature. Dotted lines—skin temperatures. Crossed line—rectal temperature. 3.25 to 3.45, carotids cooled; 4.5 to 4.45, carotids warmed.

occurred at the same brain temperature. It is naturally not easy to keep the skin temperature completely under control and in the experiment given it varied somewhat, though in another of the same type it remained so far as could be judged from two thermocouples absolutely constant. Of course heating the carotid blood caused an increase in the temperature of the skin of the head. Attempts to avoid this difficulty by heating the brain by a current of hot water passing through a double way tube into the brain tissue failed. The introduction of such a tube together with a thermopile proved too severe. That, however, skin temperature is without influence is shown by the next illustration (Fig. 3). In this the animal was heated in a covered metal box almost completely immersed in a water bath the temperature of which was varied. On heating, a vasodilation occurred and continued although on allowing the temperature of the bath to fall the skin temperature, taken by eleven thermocouples, fell far below the point at which the vasodilation started. A contraction of the blood vessels set in however as soon as the brain temperature fell to about the point at which the vasodilation had first appeared. In this

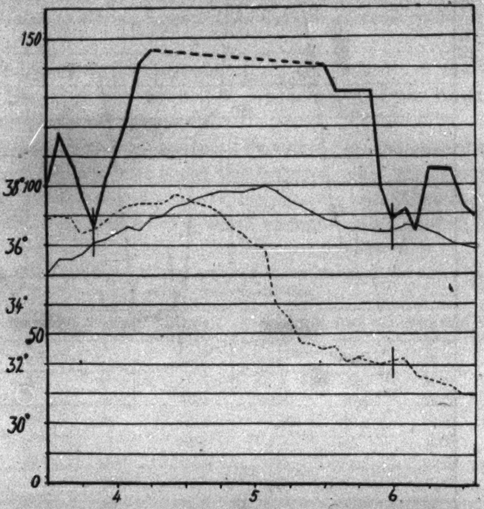


Fig. 3. Thick line—heat loss from foot. Thin line—brain temperature. Broken line—skin temperature (eleven couples in series). Bath heated from 3.45 to 4.5.

experiment the vasodilation owing to a technical difficulty could not be followed at its full height but was above the dotted line. The first vasodilation then results from the *brain temperature* passing a point fixed for the particular animal.

There is, as already stated, in animals showing this first early vasodilation a second or later vasodilation on further warming. This vasodilation shows special characters and also occurs in animals in which the first change is absent. Taking, as in the summary (Table II), all the experiments in which the animals were heated on the warmer of the table we see that while all other observations spread over relatively wide ranges of the thermometric scale, the mean skin temperature lies in almost all instances within, or very close to, the normal temperature limits (38·5°–39·5°). The average of all lies within these limits with a much smaller "mean error" than that shown by the other four columns. This

TABLE II. Showing the temperatures at the beginning of the second vasodilation in animals heated on the table.

| No. | Rectal | Brain | Skin | Skin | Mean skin |
|---|---|---|---|---|---|
| 1 | 39·7 | — | 38·4 | 39·7 | 39·0 |
| 2 | 40·3 | — | 38·1 | 41·4 | 39·8 |
| 3 | 33·9 | 31·3 | 31·2 | 46·0 | 39·6 |
| 4 | 38·8 | 35·3 | 36·7 | 42·2 | 39·5 |
| 5 | 35·9 | 35·8 | 35·2 | 42·2 | 38·7 |
| 6 | 34·8 | 34·8 | 34·3 | 42·7 | 38·5 |
| 7 | 38·3 | 37·8 | 36·9 | 42·2 | 39·6 |
| 8 | 38·4 | 39·1 | 36·9 | 45·5 | 41·2 |
| 9 | 36·0 | 37·4 | 37·5 | 42·3 | 39·9 |
| 10 | 36·4 | 35·3 | 35·4 | 39·5 | 37·4 |
| 11 | 40·2 | 34·3 | 36·9 | 42·0 | 39·5 |
| 12 | 39·0 | 36·4 | 36·6 | 42·5 | 39·5 |
| 13 | 36·2 | 36·9 | 35·5 | 42·9 | 39·2 |
| 14 | 36·8 | 36·6 | 34·3 | 43·3 | 38·8 |
| 15 | 38·6 | 35·6 | 36·2 | 42·8 | 39·5 |
| 16 | 39·2 | 37·1 | 37·3 | 41·3 | 39·3 |
| 17 | 35·8 | 37·7 | 34·2 | 38·6 | 36·4 ! |
| 18 | 39·2 | 38·0 | 38·2 | 41·7 | 40 |
| 19 | — | 37·6 | 38·3 | 43·2 | 40·7 |
| 20 | — | 37·6 | 37·8 | 40·8 | 38·8 |
| 21 | — | 38·6 | 38·1 | 42·3 | 40·2 |
| 22 | — | 37·6 | 36·7 | 41·1 | 38·9 |
| 23 | — | 39·2 | 40·2 | 41·4 | 40·8 |
| 24 | — | 37·6 | 37·8 | 43·0 | 40·2 |
| 25 | — | 39·2 | 38·5 | 44·3 | 41·1 |
|  | 37·6<br>±·9 | 36·8<br>±·7 | 36·7<br>±·7 | 42·2<br>±·5 | 39·4<br>±·2 |

coincidence and constancy seem convincing evidence of a causal relation between the skin temperature and the onset of this second vasodilation.

These and similar experiments suffer from the disadvantage that the mean skin temperature was taken as the average between two couples only which owing to the method of heating were widely different. Apart from the fact that these two might not give the correct figure for their respective sides, a smaller portion of the animal was resting on the table than exposed to the air so that their mean would be in excess of the true average.

In order to test further the significance of the skin temperature in initiating this phase of vasodilation there are three feasible methods. In the first place the vasodilation should cease when the skin temperature has come back to the normal. Secondly the dilation once started should not be stopped by lowering the brain temperature; and thirdly one might expect a proportionality between the excess of the skin temperature over the normal and the prevailing vasodilation at the time. These three methods have been tried.

The influence of the return of the skin temperature to normal could not be so easily determined as the starting of the vasodilation. The effect of heating the skin above normal, in combination no doubt with the length of the experiment, was often so severe that collapse and death resulted before the experiment could be completed. In a certain number of experiments however in which special care was taken to avoid excessive heating results were obtained. Even in these however the return to normal was usually so gradual, mainly no doubt to lag in the apparatus, that the point of reaching normal could only be approximately determined. The results are shown in the following summary. The figures in the first column indicate the corresponding experiment in the previous table. This table shows that the mean skin is the one of the five which approach nearest to the corresponding results in Table III.

TABLE III. Showing the temperatures at the end of the second vasodilation. The numbers refer to Table II.

| No. | Rectal | Brain | Skin | Skin | Mean skin |
|---|---|---|---|---|---|
| 9 | 38·5 | 38·9 | 40·3 | 41·9 | 41·1 |
| 18 | 39·6 | 36·1 | 38·1 | 39·4 | 38·7 |
| 20 | — | 37·2 | 37·8 | 41·2 | 39·5 |
| 23 | — | 39·0 | 39·9 | 41·1 | 40·5 |
| 24 | — | 37·4 | 39·4 | 42·7 | 41·0 |
| 25 | — | 39·7 | 39·4 | 41·1 | 40·2 |
|  | 39·1 | 38·0 | 39·1 | 41·4 | 40·1 |

That cooling the brain has no influence on a vasodilation started at high skin temperature is shown by Fig. 4.

The third type of experiment by which it was endeavoured to obtain additional evidence of the significance of the skin temperature in the vasodilation was of necessity more elaborate. It was hoped that by varying the skin temperature while keeping it always above the normal a corresponding variation in the intensity of the heat given off by the foot could be seen. This experiment was hampered in two directions; the blood-pressure does not remain constant, it invariably rises during the commencement of the vasodilation and then falls with the progress of the experiment and exhaustion of the animal to below the early levels. As one wishes to make the comparison as simple as possible allowance must be made for this by calculating the blood flow to be proportional to the blood-pressure. The exhaustion of the animal under the trying conditions of extreme heat shows itself however in another and incalculable fashion. Making allowance for the blood-pressure the heat loss from

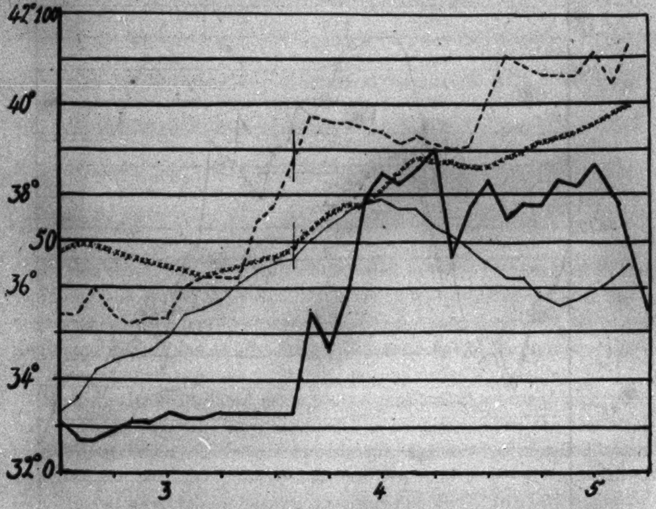


Fig. 4 (Exp. 14, Table II). Thick line—heat given off by foot. Thin line—brain temperature. Broken line—mean skin temperature. Crossed line—rectal temperature. Carotids warmed from 2.45 to 4.5 and cooled from 4.5 to 4.50.

the foot corresponding to approximately the same conditions late in an experiment is always less than those obtained earlier. As a result of these difficulties one cannot hope to be able to find an accurate correspondence in this type of experiment between the skin temperature and the heat loss from the foot, but a general relation can usually be traced. The most elaborate experiment of this type is illustrated in Fig. 5.

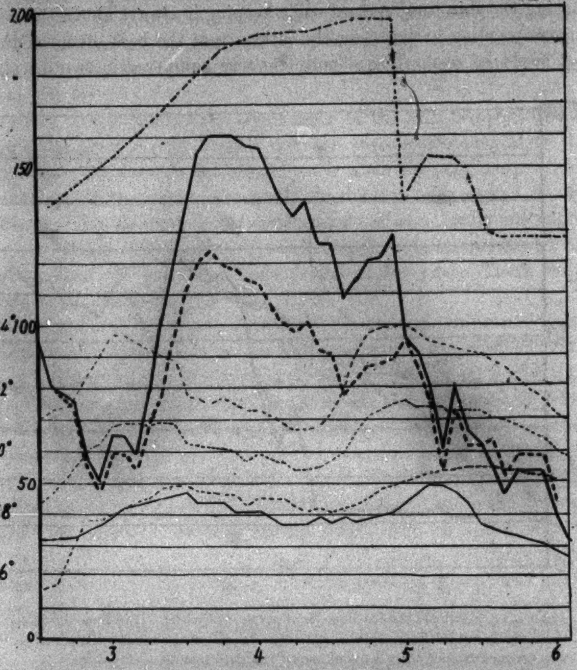


Fig. 5 (Exp. 24, Table II). Thick continuous line—heat given off leg. Thick broken line—blood flow for a B.P. of 140 mm. Thin line—brain temperature. Broken lines—skin temperature. Dot and stroke line—blood-pressure (femoral artery) in mm. Hg. Carotids cooled from 3.20 to 4, from 4.25 to 4.55 and from 5.10 to 5.45. Heating warmer stopped at 2.55, restarted at 4.25 to 4.45.

As already pointed out experiments in which the skin temperature is obtained only as an average of two points can have value only by the approximate agreement of a large number. A fresh series was consequently undertaken in which the skin temperature was taken as the mean of five points, two on each side and one on the back of the neck, and in which the animal was heated uniformly by means of the method described in the technic. The temperatures at the beginning (*b*) and end (*e*) of the second vasodilation are given in Table IV.

TABLE IV. Showing the temperatures corresponding to the beginning (*b*) and end (*e*) of the second vasodilation in animals heated uniformly.

| No. | | Brain | Average skin |
|---|---|---|---|
| 1 | *b* | 38·1 | 38·5 |
| 2 | *b* | 38·3 | 38·3 |
| 3 | *b* | 39·3 | 36·4 |
| | *e* | 37·7 | 38·4 |
| | *b* | 38·5 | 37·1 |
| 4 | *b* | 38·3 | 37·8 |
| | *e* | 38·7 | 37·9 |
| | *b* | 39·1 | 38·6 |
| 5 | *e* | 38·7 | 38·3 |
| 6 | *b* | 38·9 | 38·2 |
| | *e* | 37·8 | 38·7 |

In this table (Exp. 3 of which illustrated in Fig. 1) we see that in the beginning and end of the vasodilation the skin or brain or both are at or very near normal temperature. A question arises directly from these results and indeed comes obviously within the scope of the enquiry. Does the brain temperature alone reaching normal cause a vasodilation?

Heating the head blood alone proved unsatisfactory for this purpose but enclosing the head in a warmed box as described in the technical portion was efficient. In one experiment of this type a vasodilation appeared at a brain temperature between 38·4° and 41·2°. It disappeared on cooling at 38·8° to reappear on rewarming at 39·9°. In another similar experiment the dilation appeared at brain temperature 39·3° to disappear at 38·6° and reappear at 40·3°. While in a third the dilation was observed at 39·3° and 40°. In none of these however was there a satisfactory record of the skin temperature, but a fourth experiment showed a dilation at a brain temperature of 39·7° while the skin of the head was 37·7° and the skin of the sides 34·5° and 34·9° respectively.

We find then on warming an anæsthetised animal two marked increases in the blood flow through the leg. The earlier one occurs at a comparatively low temperature, is due to the brain temperature reaching a definite point and is uninfluenced by the skin temperature. In a previous investigation I found that chemical regulation was directed towards a subnormal point in anæsthetised animals. Whether this point coincides with the point of vasodilation could not be decided as in the absence of artificial respiration shivering was too faint to permit the determination of the exact point of its cessation. The second vasodilation occurs when the average skin or the brain temperature reaches normal showing that for purposes of physical regulation the normal temperature is much more stable than would be expected from an investigation limited to the chemical side.

This is borne out by previous investigations (5) on sweat in which it was found that sweat appeared when the brain temperature reached normal. In the same paper it was stated that when a portion of the skin reached 43° sweating also appeared. At that time I was not sufficiently aware of the importance of the average skin temperature and had hoped to control this statement from the newer point of view on this occasion. Unfortunately however owing no doubt to the anæsthetics used sweating was practically never observed.

## CONCLUSIONS.

1. The skin (of the leg) of the anæsthetised cat shows two alterations in blood flow in association with the temperature of the animal.

2. One of these depends upon the brain temperature passing a point fixed for the particular animal. It is uninfluenced by the skin temperature.

3. A second increase in flow occurs when either the skin or brain temperature reaches the normal body temperature, and disappears as soon as both have fallen below it.

## REFERENCES.

(1) **O'Connor.** Proc. Royal Soc. B, **89**. p. 201. 1916.
(2) **Rowe.** Journ. Pharm. and Exp. Ther. **9**. p. 107. 1916.
(3) **Hill** (A. V. and A. M.). Journ. of Physiol. **46**. p. 80. 1913.
(4) **Macdonald.** Proc. Royal Soc. B, **87**. p. 96. 1913.
(5) **O'Connor.** Journ. of Physiol. **49**. p. 113. 1915.

# THE SPECTROCOMPARATOR, AN APPARATUS DESIGNED FOR THE DETERMINATION OF THE PERCENTAGE SATURATION OF BLOOD WITH OXYGEN OR CARBON MONOXIDE. By AUGUST KROGH.

(*From the Laboratory of Zoophysiology, Copenhagen University.*)

THE principle of the apparatus here to be described has been suggested by the method invented by **Hartridge**(1) for determining the percentage saturation of blood with carbon monoxide. **Hartridge** employed an optical mixing for producing the spectra of definite mixtures of CO . Hb and $O_2$ . Hb, using two wedge-shaped troughs filled with CO . Hb and $O_2$ . Hb respectively and arranged in front of the slit of a spectroscope. Supposing we have one double wedge trough (*A*, Fig. 1) containing the solutions, it is apparent that if the trough is moved from 0 to 100 in front of the spectroscope we shall obtain successively the spectra of all possible mixtures of the two components, and the percentage of one of them can be at once read off from the position, if the length of the trough is divided into 100 parts. It occurred to me that if a spectroscope with comparison prism were used, and a solution containing both CO . Hb and $O_2$ . Hb in unknown proportions were examined, while a double wedge trough was arranged in front of the comparison prism and moved until the two spectra became identical, the percentage of CO . Hb in the solution to be determined could be read off directly. Similarly the percentage saturation of a Hb solution with oxygen could be read off, when the two compartments of the double wedge trough were filled with $O_2$ . Hb and reduced Hb respectively.

At first my intention was only to obtain a very rough estimate of the percentage saturation of the blood of certain invertebrates(2), and the arrangement was fitted on an Engelmann microspectroscope, but the results obtained were so promising that a more convenient and accurate apparatus was put together. This instrument, which I am now going to describe, is however very far from ideal—being made in the laboratory from an ordinary pocket spectroscope—and is to be considered only as a model, and I intend to have it improved and built by a firm of opticians as soon as circumstances will permit.

*B* 1 on Fig. 1 is the spectroscope, which has been provided with two comparison prisms (2), each occupying one-half of the slit. A small table (3) can revolve round the spectroscope and is fixed by a stop in two positions 180° apart. The table carries two grooves (4 and 5) in which glass troughs can be moved forwards and backwards. One trough is the double wedge trough mentioned above and shown diagrammatically in *A*. The lid is divided as shown, and the position of the trough

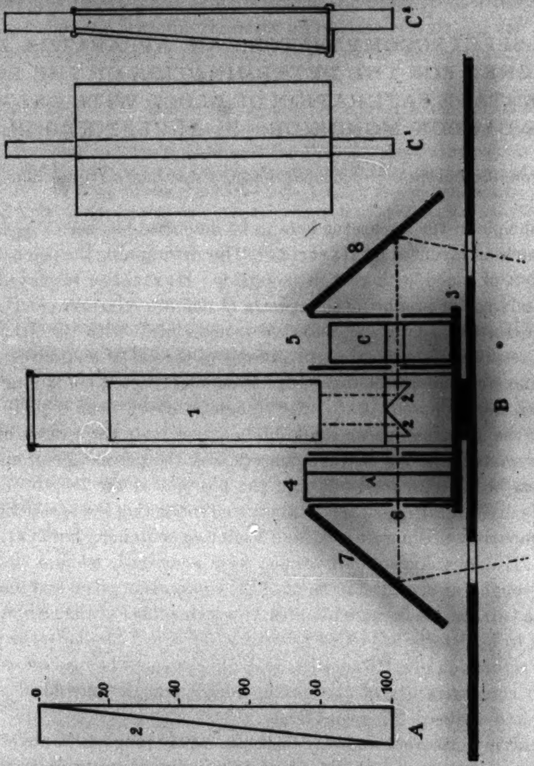


Fig. 1. The spectrocomparator.

relative to the slits by which the walls of the groove are pierced can be read off. The other trough (*C* 1 and 2) is wedge-shaped and can be filled through the tubes at both ends with the solution to be examined. It holds only 0·05 c.c. of fluid.

The apparatus is placed on a box containing an incandescent lamp and a colour filter to screen off the unnecessary portions of the spectra. The light coming up through two holes in the box is reflected by the mirrors 7 and 8 on to the solutions and the spectroscope.

When estimations of $O_2$ . Hb have to be made a solution of ox blood in Barcroft's ammonia (3) of suitable strength (usually about 5 c.c. blood to 75 c.c. ammonia) is prepared and divided in two portions. One of these is saturated with air and the other reduced by the addition of a slight excess of dry sodium hydrosulphite, and the compartments in the trough *A* are filled (1) with the $O_2$ . Hb solution, and (2) with the reduced. The solution to be determined is sucked into the trough *C*.

In making the estimation *C* is first brought into such a position that the total absorbing powers of the solutions to be compared are about equal, that is the concentrations are inversely proportional to the thickness of the layer, which remains constant in *A* but is altered by the movement in *C*; thereupon *A* is moved forwards and backwards until the two spectra are identical. The percentage saturation is read off and the process repeated after reversing the table (3). Usually six consecutive estimations, which can be performed in less than five minutes, have been averaged.

The agreement between the single estimations in such series is shown in the following examples which have been selected at random.

| | Left | Right | L. | R. | L. | R. | L. | R. |
|---|---|---|---|---|---|---|---|---|
| Readings | 15 | 14 | 37 | 35 | 51 | 49 | 67 | 68 |
| | 12 | 13 | 36 | 34 | 50 | 51 | 66 | 69 |
| | 15 | 14 | 36 | 36 | 51 | 48 | 69 | 69 |
| Averages | 14 | 13·7 | 36·3 | 35 | 50·7 | 49·3 | 67·3 | 67·3 |
| | 14 | | 35·7 | | 50 | | 67·3 | |

The method has been tested in the following manner. A portion of blood was completely reduced by bubbling in vacuum with pure nitrogen, while another was oxidised. A pyknometer holding 10 c.c. was filled with oxygen free Barcroft's ammonia (and a few glass beads for mixing) and blood solutions with definite $O_2$ saturations prepared by adding measured volumes of oxidised and reduced blood. After mixing, a little of the solution was introduced into the wedge *C* and the percentage saturation determined as described above. The saturations prepared were in all cases unknown to the person making the estimations.

The results of the first series of control determinations were as follows:

| % saturation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| prepared | 66·7 | 34·5 | 50 | 66·7 | 80 | 33·3 | 20 | 26·5 | 40 | 60 | 20 | 66·6 | 40 | 66·6 |
| estimated | 50 | 36·5 | 52·5 | 68 | 71 | 36 | 27 | 30·5 | 41 | 60·5 | 33·5 | 67·5 | 42·5 | 69 |

It was found that at very low $O_2$ saturations and especially at high (above 70 per cent.) the determinations become unreliable, because the spectrum changes too slowly. I have no doubt, however, that with a better spectroscope this difficulty can be overcome.

For the purpose of making determinations of the percentage saturation on very small samples of blood the following device was adopted. A glass tap was made (Fig. 2) having instead of a boring a small pocket taking 0·2 c.c. This is filled with oxygen free Barcroft's ammonia and closed. When the blood is ready in a capillary pipette it is slightly opened and 20–50 mm. blood introduced. It is again closed and shaken and thereupon the small wedge *C* is filled from it by sucking.

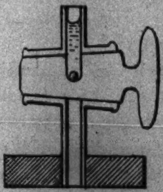


Fig. 2. Tap for preparing blood solutions in the absence of air.

This procedure, together with the actual estimations, was tested and compared with saturation determinations according to Barcroft(3) (½ c.c. samples). Definite mixtures of oxidised and reduced blood were prepared in a pyknometer and samples taken from these both for Barcroft determinations and for solution in the mixing tap and subsequent determination by means of the spectrocomparator. The results, which are shown graphically in Fig. 3, were as follows:

| % saturation | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| prepared ... ... | 11·5 | 15 | 15 | 21 | 25 | 34 | 40 | 40 | 45 | 50 | 50 | 52 | 65 | 70 | 75 | 84 |
| estimated | | | | | | | | | | | | | | | | |
| by spectroscope | 14 | 16 | 20 | 20 | 29 | 36 | 40 | 40 | 44 | 50 | 53 | 50 | 65 | 67 | 70–100 | 70–100 |
| by Barcroft's method ... | | | (6·5) | 19 | | | 37 | | | | 50 | 51 | | 66 | | 82 |

A series of determinations have been made on definite mixtures of CO . Hb with $O_2$ . Hb. The results were as follows:

| % saturation with CO | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| prepared ... | 10 | 13·3 | 13·3 | 20 | 20 | 20 | 20 | 33·3 | 33·3 | 33·3 | 40 | 40 | 50 | 60 | 66·7 |
| estimated by spectroscope | 14 | 12 | 15 | 20 | 21 | 26·5 | 22 | 33 | 37 | 38 | 42 | 41 | 51 | 60 | 67 |

The determinations proved to be difficult, because the difference between the two spectra is so small, but also in this case I believe that much better results will be obtained with a more perfect instrument, especially with a spectroscope giving a greater dispersion.

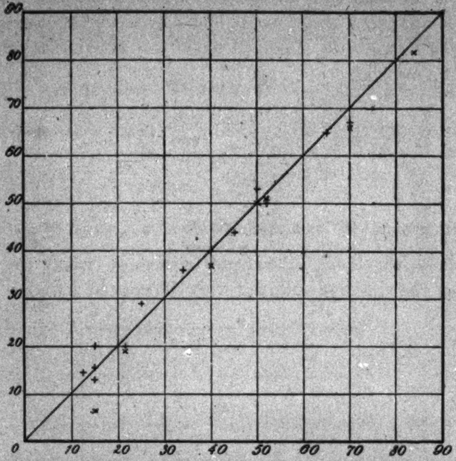


Fig. 3. Percentage saturation of blood with oxygen. Spectrocomparator results (+) compared with Barcroft determinations (×). Abscissa percentage saturations prepared.

The spectrocomparator method has been used for a number of determinations on the blood of fishes (4). A special and very serious difficulty was encountered in this case: the corpuscles cannot be properly dissolved, neither with ammonia nor in any other way so far discoverable. Solution is always accompanied with clotting of the blood or part of it into a jelly. We were compelled therefore to see whether it might be possible to work on genuine defibrinated or oxalate blood. The undiluted blood was subjected to estimation in a coverslip wedge made by putting a thick coverslip on to a slide and forming a wedge by means of a second coverslip, Fig. 4. Into such a wedge the blood was introduced directly from a small pipette, and though it became rapidly oxidised along the open edges it kept sufficiently long in the middle to allow the spectrocomparator readings to be taken. Used in this way

Fig. 4. The coverslip wedge.

the spectrocomparator is much less accurate than with dissolved blood, and we found, moreover, that the results show systematic variations from the true saturations, and that these are different for the blood of different animals. The details of these results will be given in a subsequent paper by **Miss Leitch** and the writer (4), and here I give only a series of comparisons made on ox blood between the coverslip wedge, the ordinary wedge with dissolved blood and determinations according to Barcroft's method.

| % saturation with $O_2$ | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| By Barcroft's method | 21<br>22 | 23 | 24<br>28 | 28·5 | 28<br>30 | 32·5 | 32<br>35 | 44·5 | 43·5<br>47 | 50 | 50·5<br>52·5 | 50·5<br>53 | 58 | 65·5 | 66<br>67 | 73·5<br>75·5 |
| By spectro-comparator: | | | | | | | | | | | | | | | | |
| dissolved blood | | 29 | | | | 37 | | 40 | | 55 | | | 60 | 67 | | |
| coverslip wedge | 21 | 25·5 | 22 | 19 | 28·5 | 28·5 | 31·5 | 32·5 | 29 | 38 | 37·5 | 42·5 | 42 | 50 | 45 | 50 |

The results which are plotted in Fig. 5 show clearly the systematic deviation of the readings obtained with the coverslip wedge.

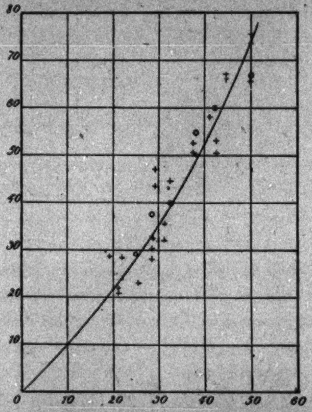


Fig. 5. Calibration of coverslip wedge. Abscissa spectrocomparator readings with coverslip wedge. Ordinates true saturations as determined by Barcroft's method (+) or by spectrocomparator on dissolved blood (○).

In all the comparative tests mentioned, I have been assisted by Miss **I. Leitch** and Mr **Gad-Andresen**, to both of whom my thanks are due.

The expenses of the research have been partly defrayed by a grant from the Carlsberg foundation.

## SUMMARY.

A preliminary description is given of a spectrocomparator in which the spectra of solutions containing $O_2 . Hb$ and Hb or $O_2 . Hb$ and CO . Hb can be directly matched with the spectra of the two components, combined so as to allow the direct reading of the percentage saturation of the solution examined.

A series of experiments to test the spectrocomparator are described.

## REFERENCES.

(1) **Hartridge.** Journ. of Physiol. **44.** p. 1. 1912.
(2) **Leitch.** Journ. of Physiol. **50.** p. 370. 1916.
(3) **Barcroft.** The Respiratory Function of the Blood. Cambridge, 1914. Appendix.
(4) **Krogh and Leitch.** Journ. of Physiol. **52.** p. 288. 1919.

# THE RESPIRATORY FUNCTION OF THE BLOOD IN FISHES. By A. KROGH and I. LEITCH.

(*From the Laboratory of Zoophysiology, Copenhagen University.*)

THE influence of the temperature upon the dissociation curve of oxyhæmoglobin has been investigated by Barcroft and A. V. Hill(1), and their results are summarised in the curves given by Barcroft(2), which show that the rectangular hyperbolas, which represent the relation between oxygen tension and percentage saturation with $O_2$ of a solution of hæmoglobin, are much increased in steepness by decreasing temperature. The relations between the temperature and the dissociation curves of Hb as determined by Barcroft and Hill are given by the formulæ

$$(1)\ \frac{y}{100}=\frac{Kx}{1+Kx} \quad \text{and} \quad (2)\ \frac{K_2}{K_1}=e^{-\frac{q}{2}\cdot\frac{T_2-T_1}{T_2T_1}}$$

of which the first is the equation of a hyperbolic dissociation curve and the second expresses the relations of the constants of two dissociation curves valid at the absolute temperatures $T_2$ and $T_1$. In (1) $y$ is the percentage saturation of the Hb with oxygen and $x$ the oxygen pressure in mm. Hg. In (2) $q$ is the heat evolved when one gram-molecule of oxygen combines with one gram-molecule of Hb. $q$ has been determined by Barcroft and Hill as 28,000 gram calories and shown to be constant over a range of temperatures from 16° to 49°. In the following we have assumed it to be constant also below 16°. Barcroft and Roberts(3) determined the value of $K$ at 38° as 0·125. From these data a curve for the dissociation of oxyhæmoglobin can be calculated for any temperature. Each hyperbola is characterised by the value of $K$. We have found it much more convenient however to use $\frac{1}{K}$ as a characteristic, since it can be easily shown that $\frac{1}{K}$ corresponds to the $O_2$ pressure at which the Hb is just half saturated with oxygen[1]. This $O_2$ pressure we denote as the

---

[1] We have at the point of half saturation $y=50$ and hence from (1)

$$\frac{50}{100}=\frac{Kx}{1+Kx} \text{ or } x=\frac{1}{K}.$$

"tension of unloading" of the Hb $t_u$, since normally the circulating blood can be taken to give off about one-half of its oxygen to the tissues. $t_u$ can be used as a single though incomplete characteristic also of the dissociation curve of genuine blood. For the hyperbolic curves of pure Hb solutions we have calculated the following figures:

| Temperature | $1/K = t_u$ mm. |
|---|---|
| 38° | 8·00 |
| 30 | 2·43 |
| 25 | 1·12 |
| 20 | 0·503 |
| 15 | 0·218 |
| 10 | 0·092 |
| 5 | 0·038 |
| 0 | 0·015 |

These show clearly that at low temperatures pure Hb must be very badly adapted for oxygen carrying purposes, since the pressures at which it parts with any considerable portion of its combined oxygen become extremely low.

Barcroft and his collaborators have shown that in the organism of mammals the substances present along with the Hb in the corpuscles and especially the electrolytes and the hydrogen ions have a very marked influence on the affinity of the hæmoglobin for oxygen, modifying the curve in such a way that the tension of loading is reduced, while the tension of unloading is raised[1].

The blood is therefore better adapted for its function of carrying oxygen from the respiratory organs to the tissues than a solution of pure Hb would be. If we take the tension of loading ($t_l$) as corresponding to 95 per cent. saturation we have for average human blood (at 38°) $t_l = 85$ mm. and $t_u = 27$ mm. while for pure Hb the corresponding figures are $t_l = 152$ mm. and $t_u = 8$ mm.

The problem studied in the present paper is the adaptation of the blood of fishes to the transport of oxygen, an adaptation which must *a priori* be thought to be very necessary and at the same time extremely difficult to obtain, when we consider that at a temperature of 10°, which is high for most fishes, the $t_u$ of pure Hb is less than $\frac{1}{10}$ mm. oxygen pressure.

*Methods.* The principles on which our methods have been based are identical with those introduced by Bohr and since employed by all experimenters. We expose samples of the blood in question in saturators[1] to atmospheres of known pressures and compositions and maintain a definite constant temperature during the process. When equilibrium has been reached part of the blood is withdrawn and its percentage saturation with oxygen determined. As saturators we have in a number of experiments used Barcroft's model (2), but more often we have employed a small glass bulb of 2 c.c. capacity through which a current of the required gas mixture could be maintained. Our method of filling saturators with gas and generally of preparing definite gas mixtures for saturation purposes is the following. We prepare a stock of pure nitrogen, which we keep in gasometer bottles of 8 l. capacity over a solution of sodium hydrosulphite. The solution is protected in the open bottle by a thick layer of paraffin oil and a fluid seal is inserted in the cork. When a definite $O_2$ pressure is to be provided in a saturator or other vessel it is first filled with pure outside air (20·95 per cent. $O_2$) and then connected with a filter pump and a vacuum meter and exhausted until the desired $O_2$ pressure is reached. Thereupon the vessel is filled with pure nitrogen from the gasometer. When $CO_2$ has to be added this is done afterwards from a gas burette or glass syringe, and when the volume of the vessel has been determined beforehand it is easy to obtain a definite $CO_2$ pressure. This method of filling has been checked in a number of cases by analysis and found to be very reliable. Consequently it has been used also at such low oxygen pressures that analysis could not give a sufficiently accurate determination of the oxygen.

---

[1] In the formula for the dissociation curve of genuine blood $\frac{y}{100} = \frac{Kx^n}{1+Kx^n}$ the tension of unloading $t_u$ does *not* correspond to $\frac{1}{K}$ but, as can be easily shown, to $t_u = \left(\frac{1}{K}\right)^{\frac{1}{n}}$.

The determination of the percentage saturation of fish blood with oxygen is very difficult, and a completely satisfactory method has not been found. The pump could of course be employed, but the small quantity of blood available (about 10 c.c. from a fish of 1–2 kg.) and the low oxygen capacity would constitute a serious drawback. The ordinary ferricyanide method cannot be used at all, since the blood cannot be laked and a solid clot will always be formed on adding ferricyanide. We have satisfied ourselves that a colorimetric determination of the oxygen capacity after Haldane will give reliable results on fish blood by reducing the blood of different fishes (carp, cod) and then measuring the quantity of oxygen taken up from atmospheric air in Barcroft's differential blood gas apparatus and comparing these measurements with the $O_2$ capacities of the blood samples as determined colorimetrically.

---

[1] Apparatus intended for saturating blood with gases at definite tensions should be called saturators and not "tonometers" or "ærotonometers." This latter name has been given originally to instruments arranged for the determination of gas tensions possessed by a fluid on entering the apparatus.

Oxygen capacities of cod blood c.c. $O_2$ per litre blood.

| Absorption method corrected for physical absorption | Hæmoglobinometer | Difference |
|---|---|---|
| 55 | 56 | +1 |
| 100 | 98·5 | −1·5 |
| 104 | 107 | +3 |
| 104·8, 105·8 | 98 | −7 |
| 83·2, 83·8 | 80 | −3·5 |
| 95, 84 | 86 | −3 |
| 83·4, 83·2 | 81 | −2 |

The hæmoglobinometer used had been calibrated on ox blood by means of the blood gas apparatus and the ferricyanide method. The calibration was checked during the determinations given above and absolutely confirmed ($O_2$ capacity by ferricyanide 187·0, 188·6, average 187·8, by hæmoglobinometer 187·5).

The oxygen capacity of the blood of the different fishes examined is generally about 80 to 100 but lower values are rather often observed, and two of the fishes examined (one cod, one plaice) were distinctly anæmic ($O_2$ capacity 17–20).

Relying on these results we have made a number of determinations of the percentage saturation with oxygen of ½ c.c. blood samples from the saturator by measuring the amount of $O_2$ taken up from air in the differential blood gas apparatus and dividing by the total $O_2$ capacity as determined colorimetrically. It is necessary in these determinations to protect the blood from contact with the air by means of saline instead of Barcroft's ammonia[1]. Otherwise the blood will clot. We found that when the blood had been exposed in the saturator to a measurable tension of $CO_2$ this method could not be used, that is to say the manometer of the differential apparatus could not be brought to a standstill. In these cases our only possible method was the determination by means of the spectro-comparator and the coverslip wedge (4). As mentioned in the preceding paper by A. Krogh the coverslip wedge has the very serious drawback that the readings obtained do not always show directly the percentage saturation of the blood samples, but have to be corrected according to comparisons with results obtained by a direct method. A series of such comparisons were made on the blood of the cod, plaice and trout with the following results:

---

[1] We have put weak alkali into the ferricyanide reservoir to maintain the $CO_2$ pressure at 0.

Percentage saturation of blood.

| | Cod | | | | | | Plaice | | | | Trout | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spectro-comparator coverslip wedge | (39) | 42·5 | 52·5 | 29 | 31 | 30·5 | 43·5 | 26·5 | 15 | 52·5 | 33·5 | 54·5 |
| Blood gas apparatus absorption method ... ... | 47 | 75 | (100)[2] | 45·5 | 44·5 | 52 | 76·5 | 53 | 33 | (100)[1] | 58·5 | (100)[1] |

These results are plotted in Fig. 1 with the spectro-comparator readings as abscissa and the true saturations as ordinates, and it is seen that the curve drawn satisfies them tolerably well. For the three species named this curve has therefore been employed to reduce the spectro-comparator readings to true oxygen saturations.

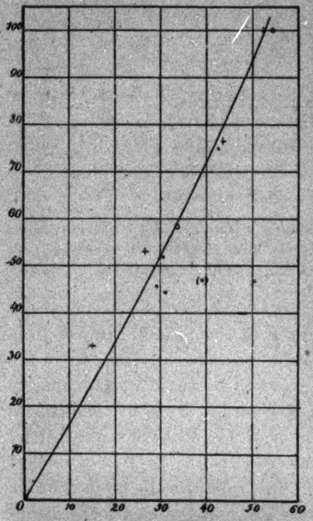


Fig. 1. Calibration of coverslip wedge for fish blood. Abscissa spectro-comparator readings. Ordinates true saturations as determined by absorption. ● determinations on cod, + on plaice, ○ on trout.

In the case of the other fishes examined, carp, pike and eel, no systematic difference between the spectro-comparator and the absorption determinations could be made out, though the saturations above 50 per cent. determined by means of the former instrument are probably too low, since blood saturated with air appears to be only 65 per cent. saturated.

It is evident that very accurate results cannot be expected from the methods described, and it has not been attempted therefore to subject the material to mathematical treatment. We have only had in view the purely biological problem: How far is the blood of different fishes adapted to the oxygen carrying function?

*Fresh water fishes.* We have examined the blood of the carp, pike and eel, which proved to be very similar, and give as an example the results obtained on the carp.

---

[1] The blood was saturated with atmospheric air.

| Tp. | $O_2$ tension mm. | $CO_2$ tension mm. | Saturation by spectro-comparator % | Saturation by absorption % |
|---|---|---|---|---|
| 17° | 0·7 | 0·3 | 35 | 27 |
| — | 1·0 | (0·3) | 32 | 30·5 |
| — | 2·0 | 0·3 | 42 | 40 |
| — | ? | (0·3) | 52 | 56 |
| — | 150 | (0·3) | (65) | (100) |
| — | 2·5 | 7·5 | 29 | |
| — | 2·5 | 7·5 | 27·5 | |
| 15° | 2 | 0·3 | 47 | |
| — | 25 | 15 | 37 | |
| — | 150 | 15 | 52·5 | |

The $CO_2$ tensions given as (0·3) have not been measured, but according to the results of a few determinations it has been assumed that a tension of this value would generally be produced when the blood was treated with atmospheric air or mixtures of air with pure nitrogen. The inevitable variations in this low tension may possibly have a greater influence than we thought while doing the experiments and be responsible for some of the discrepancies encountered.

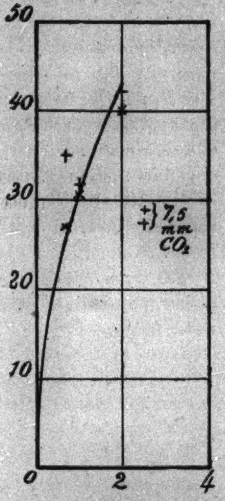


Fig. 2. Percentage saturation of carp blood with oxygen at 17°. Abscissa $O_2$ tension in mm. Ordinates saturation per cent. + determinations by spectro-comparator, × determinations by absorption.

The results obtained at 17° are given in the curve Fig. 2. At 17° the $t_u$ is about 3 mm. while for pure hæmoglobin it is 0·3 mm. only. We find moreover that the effect of $CO_2$ on the dissociation is very considerable.

The figures found for the pike are similar to those given for the carp, and the curves of the $CO_2$ free blood seem to be practically identical. A very marked effect of $CO_2$ is found as in the carp.

The same holds good also for the blood of eels, though the results obtained on different specimens appear to be rather discordant.

The $O_2$ dissociation curves of the blood of the three fishes are very steep. At 15° the $t_1$ is about 10 mm. and the $t_u$ of the $CO_2$ free blood about 2–3 mm. but a $CO_2$ tension of 1 per cent. (7·5 mm.)

will raise the $t_u$ to at least 5 mm. and probably more. It is evident that the low loading tension of the blood will make the fishes well adapted to withstand low oxygen pressure in the water and it is very significant that Winterstein (5) found that fresh water fishes (Leuciscus) do not begin to suffer from want of oxygen until the dissolved quantity of $O_2$ in the water falls below 0·6 c.c. per litre corresponding to a tension of 14 mm. at 15°. Recently Gaarder (6), working in this laboratory, has obtained similar results on the carp. In lakes and ponds, but not in running water, the $O_2$ tension is very variable and has been observed to sink to very low values. The low loading tension of the blood is therefore a very important adaptation to the biological conditions.

It might be argued that the large amount of $CO_2$ which must be formed, when the oxygen in a lake is reduced to a very low tension, must lower the dissociation curve and inhibit the loading of the blood, but in natural waters (of lakes and ponds) the $CO_2$ tension never rises beyond 1 or 2 mm. (Krogh (7)) and is generally much lower owing partly to the great solubility of $CO_2$ in water, partly to the almost universal presence of substances, such as $CaCO_3$, which combine with $CO_2$ and possess a very low tension of dissociation (7). The influence of the $CO_2$ produced in the metabolic processes is therefore practically confined to the capillaries in which it will greatly facilitate the unloading of the oxygen from the blood.

*Sea water fishes.* We have examined the blood of the cod and the plaice and obtained results very different from those found on the fresh water fishes.

$O_2$ dissociation of cod blood.

| | | | Percentage saturation by | | |
|---|---|---|---|---|---|
| Tp. | $O_2$ tension mm. | $CO_2$ tension mm. | spectro-comparator | corrected + | absorption × |
| 14° | 15 | (0·3) | 29 | 51 | 45 |
| — | 15 | — | 31 | 54 | 46 |
| — | 15 | — | (39) | — | 47 |
| — | 15 | — | 30·5 | 53 | 52 |
| — | 26 | — | 42·5 | 77 | 75 |
| — | 150 | — | 52·5 | 100 | 100 |
| — | 150 | — | 51 | 96 | |
| — | 150 | 7·5 | 41 | 79 | |
| — | 25 | 15 | <10 | <14 | |
| — | 150 | 15 | 19 | 32·5 | |

$O_2$ dissociation of plaice blood.

| Tp. | $O_2$ tension mm. | $CO_2$ tension mm. | Percentage saturation with $O_2$ by | | |
|---|---|---|---|---|---|
| | | | spectro-comparator | corrected + | absorption × |
| 16–17° | 2·6 | (0·3) | 11 | 18·5 | |
| — | 7 | — | 15 | 25·5 | 33 |
| — | 10 | — | 27 | 47 | |
| — | 12 | — | 27 | 47 | 56 |
| — | 16·5 | — | 36 | 64 | |
| — | 16·5 | — | 30 | 52·5 | |
| — | 20 | — | 44 | 80 | |
| — | 20·5 | — | 44 | 80 | 87 |
| — | 21 | — | 43 | 78 | |
| — | 21 | — | 42 | 74 | |
| — | 27 | — | 44 | 80 | |
| — | 31 | — | 54 | 103 | |
| — | 150 | — | 53 | 100 | |
| — | 25 | 4 | 26 | 45 | |
| — | 40·5 | 6 | 36 | 64 | |

The principal results are shown graphically in Figs. 3 and 4. It is seen that at temperatures of 14° and 16°–17° respectively the $O_2$ dissociation curves of the cod and the plaice are not so steep even as the curve of pure Hb at 38° and not much steeper than the blood curve of man corresponding to a $CO_2$ pressure of 40 mm. The influence of $CO_2$ (hydrogen ions) is very considerable as in the fresh water fishes. The loading tension ($t_l$) at 15° is probably about 70 mm. for the cod and 40 for the plaice while the tension of unloading ($t_u$) at 15° is about 18 mm. for the cod and 10 for the plaice when the $CO_2$ pressure is negligible. Under the influence of the $CO_2$ tension which must exist in the capillaries the $t_u$ must be raised to figures not much, if at all, lower than the $t_l$. The $O_2$ pressure head available for diffusion into the tissues from the capillaries must therefore be higher even than in mammals. On the other hand it follows from the high loading tension that the fishes are not well adapted to stand low $O_2$ pressures or high or even moderate $CO_2$ pressures in the surrounding water.

In sea water the oxygen is practically always present in abundance. At all depths in the ocean and likewise in the narrow seas the water is never very far from being saturated with $O_2$, and the tension is therefore well above the 70 mm. at which a cod would (at 15° temperature) begin to suffer from oxygen want. The only exceptions to this rule are some very limited areas with considerable depth, in which the water can be shut off from the general circulation, and in such holes fishes are absent.

The $CO_2$ tension of sea water is always extremely low, a fact which is easily understood when we consider that even if the total possible amount of dissolved oxygen (7 c.c. per litre) were converted into $CO_2$ this would only increase the tension from 0·2 to 1 mm. (7).

It has long been known that sea water fishes are very sensitive to oxygen want, and the fact has been hypothetically accounted for in various ways. It seems clear that the form of the $O_2$ dissociation curve of their blood must contribute very markedly to this sensitiveness, which makes the maintenance of sea water aquaria so much more difficult than fresh water aquaria.

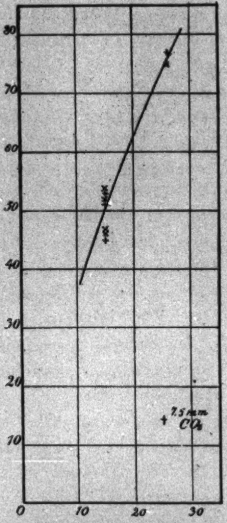


Fig. 3. Percentage saturation of cod blood with oxygen at 14°. + determinations by spectro-comparator (corrected), × determinations by absorption.

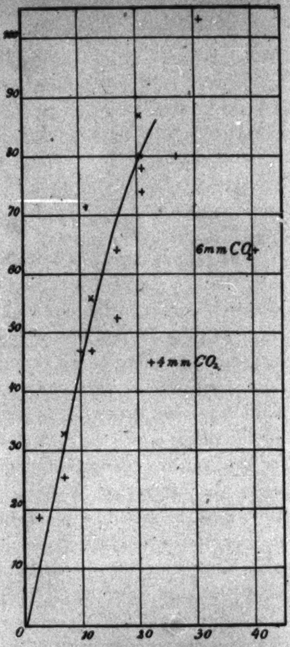


Fig. 4. Percentage saturation of plaice blood with oxygen at 16°–17°.

The difference in curve between a bottom fish like the plaice and a high sea fish like the cod cannot be teleologically accounted for by the water in or near the bottom containing less oxygen as the difference—if any—is very small, but it may well be that the mechanical ventilation of the gills is so much hindered in a fish lying partially buried in the bottom that it becomes essential for it to be able to utilise the oxygen down to a lower tension.

In order to check our contention that the oxygen dissociation curve of the blood in fishes is adapted to the oxygen supply of their natural environment we have examined the blood of the trout as a fish which, though living in fresh water, never suffers itself to be exposed to low oxygen pressures and is known to be easily killed when the water is not properly renewed or ærated. We obtained the following results which bear out our contention:

| | | | Percentage saturation by | | |
|---|---|---|---|---|---|
| Tp. | $O_2$ tension mm. | $CO_2$ tension mm. | spectro-comparator | corrected + | absorption × |
| 17° | 9 | (0·3) | 18 | 31 | |
| — | 13 | — | 26 | 45 | |
| — | 13 | — | (38) | (68) | |
| — | 13·5 | — | 24 | 42 | |
| — | 13·5 | — | 22 | 38 | |
| — | 13·5 | — | 22 | 38 | |
| — | 15·5 | — | 33·5 | 59 | |
| — | 15·5 | — | 35 | 62 | |
| — | 18 | — | 35 | 62 | |
| — | 23 | — | 44 | 81 | |
| — | 30 | — | 55 | (105) | |
| — | 150 | — | 55 | 105 | |
| — | 13 | 6 | 17 | 38 | |
| — | 19 | 4·5 | 27 | 47 | |
| 14° | 15 | (0·3) | 34 | 59 | 59 |
| — | 150 | (0·3) | 55 | 105 | |

The curve, Fig. 5, is similar to that of the plaice and differs widely from those of the other fresh water fishes examined. The influence of $CO_2$ upon the dissociation of $O_2$ Hb appears to be smaller than in the other fishes though still very considerable.

In the following small table we have given the $t_u$ of the $CO_2$ free blood of the different fishes as observed, and attempted a reduction of the figures to a common temperature of 15°. This has been done on the assumption that the variation of $t_u$ with the temperature is proportional to that of pure hæmoglobin where $t_u = \frac{1}{K}$. This assumption is probably incorrect, but the error involved is not likely to be large. In the cases of the carp and the pike where we have determinations at two different temperatures from which to make the calculation we have obtained practically the same results from both.

| | Carp | | Pike | | Eel | Trout | Plaice | Cod |
|---|---|---|---|---|---|---|---|---|
| Temperature | 17° | 15° | 18° | 11° | 17° | 17° | 16·5° | 14° |
| $t_u$ observed, mm. | 3 | 2 | 3·5 | 1·0 | (4) | 15 | 12 | 15 |
| $t_u$ at 15° calculated, mm. | 2·2 | 2·0 | 2·0 | 2·3 | (2·4) | 11 | 10 | 18 |

On the possible mechanism by which the adaptation of the fish blood to the available supply of oxygen is brought about we can at present only offer vague suggestions. *A priori* an acid reaction of the blood would present the simplest explanation of the increase in $t_u$ above that of pure hæmoglobin, but if the blood were acid it would, in our opinion, be very slightly if at all affected by an increase in the $CO_2$ tension which could not appreciably increase the acidity, and we have found that the blood of all the fishes examined is very sensitive to small increases in $CO_2$ tension which reduce the affinity for oxygen very considerably.

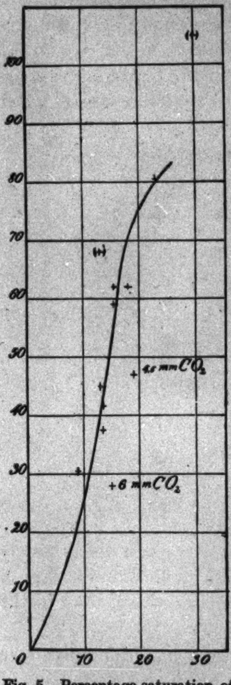


Fig. 5. Percentage saturation of trout blood with oxygen at 17°.

The clotting of defibrinated fish blood on laking points to the existence within the corpuscles and perhaps in combination with the hæmoglobin of proteins which have no equivalent in the warm-blooded organism. Nothing is known, however, about differences in the globin portion of the Hb molecule having anything to do with differences in the dissociation curve. Our experiments show that fish hæmoglobin has the same spectrum as that of mammals and, moreover, that there is the same quantitative parallelism between the colour of a blood solution and its total oxygen capacity.

We believe that the adaptation of the fish blood must be brought about by some substance or substances present along with the hæmoglobin within the corpuscles, and we wish to point out the general significance of the hæmoglobin being enclosed in corpuscles surrounded by semipermeable membranes. By this arrangement just that chemical environment can be secured which is most suitable for the respiratory function of the hæmoglobin in that particular organism, while at the same time the chemical composition of the blood plasm can be adapted, as it must needs be, to the general requirements of the body cells. If the Hb were simply dissolved in the blood plasm a conflict between the two adaptations, which may both be absolutely essential, would be inevitable, and the possession of semipermeable red corpuscles is therefore a necessary condition for utilising to the full the wonderful respiratory properties of the hæmoglobin. A hæmoglobin dissolved in blood plasm can in a cold-blooded animal only be useful at very low oxygen pressures, and that is probably why in invertebrates the possession of hæmoglobin is restricted to forms which are habitually exposed to such pressures, as pointed out by one of us (8).

The expenses of the research have been partly defrayed by a grant from the Carlsberg foundation.

## SUMMARY.

Experiments are described to determine the $O_2$ dissociation curves of the blood of fishes at temperatures between 10° and 20° and with and without the presence of $CO_2$. The tension at which the blood is half saturated with oxygen is defined as the tension of unloading $t_u$ of the blood.

In fresh water fishes (carp, eel and pike) which are occasionally exposed to low oxygen pressures the $t_u$ in the absence of $CO_2$ is comparatively low (2–3 mm. at 15°), though much higher than in pure hæmoglobin, but it becomes greatly increased by $CO_2$ tensions of 1 per cent. (7·5 mm.) or less.

In sea water fishes (cod and plaice) and in such fresh water fishes as the trout, which normally are never exposed to very low oxygen pressures, the $t_u$ of the $CO_2$ free blood at 15° is nearly as high as in mammals at 37°, viz. 18 mm. in the cod and 10–11 in the plaice and trout, and in these also the presence of a small amount of $CO_2$ diminishes very considerably the affinity of the blood for oxygen.

The dissociation curve is in both cases considered as specially adapted to the biological conditions.

The general significance of the hæmoglobin being present in semipermeable corpuscles and not simply dissolved in the plasma is ascribed to the fact that this arrangement makes possible the adaptation of the

hæmoglobin to extremely different respiratory conditions without interfering with the general composition of the blood.

## REFERENCES.

(1) **Barcroft and Hill.** Journ. of Physiol. **39**. p. 411. 1909.
(2) **Barcroft.** The Respiratory Function of the Blood. Cambridge. 1914.
(3) **Barcroft and Roberts.** Journ. of Physiol. **39**. p. 149. 1909.
(4) **Krogh.** Ibid. **52**. p. 281. 1919.
(5) **Winterstein.** Pflüger's Archiv, **125**. 1908.
(6) **Gaarder.** Bioch. Zeitschr. **89**. 1918.
(7) **Krogh.** $CO_2$ tension of natural waters. Medd. om Gronland, **26**. 1904.
(8) **Leitch.** Journ. of Physiol. **50**. p. 370. 1916.

# ADRENALIN AND PITUITRIN—A STUDY IN INTERACTION AND INTERRELATION. BY DOUGLAS COW.

*(From the Pharmacological Laboratory, Cambridge.)*

THE action of extracts of the posterior lobe of the pituitary body in increasing the tonus and movements of the uterus, as described by Dale(1), is well recognized. Similarly there is general agreement that extracts of the medullary portion of the suprarenal gland produce that effect on the uterus which is obtained by stimulation of the hypogastric nerve. In certain animals, such as the rabbit, the uterus always responds to adrenalin by contraction(2): in another class, represented by the cat, the virgin (or non-pregnant) uterus is inhibited whilst the pregnant organ is contracted by adrenalin(3): in yet a third class, including the guinea-pig and the rat, the effect of adrenalin is to inhibit uterine movements whether the organ is pregnant or not(4). The brothers Gunn(4) give in tabular form the reactions to adrenalin of the uterus of the usual laboratory animals and of certain others, culled from previous literature.

The explanation usually given to account for these differences is that motor fibres (or nerve-endings) predominate over inhibitor in the hypogastric nerves of those animals in which the uterus is contracted by adrenalin, and that the reverse holds good in those animals in which adrenalin inhibits the uterus, whilst in the case of the cat it is supposed that the proportion of motor and inhibitor fibres is so evenly balanced that a hypothetical greater increase in the motor fibres than in the inhibitor during pregnancy is sufficient to change completely the reaction to adrenalin of the uterus.

Whilst these broad statements as to the qualitative reaction of the uterus to adrenalin hold good no doubt in most cases, one occasionally finds a uterus which responds to adrenalin in what must be considered an abnormal way, if one subscribes to the theory of preponderance of motor over inhibitor fibres (or *vice versa*) as the sole determining factor. I have on occasion observed such an "abnormal" response in the case of the guinea-pig's uterus, and have recorded such a response in a previous communication(5), though it has been suggested to me since then that the mere fact that the uterus responded by contraction in this instance is sufficient to show that, whatever else may have caused the contraction, it was not adrenalin.

Further if one postulates this preponderance of one type of nerve-ending over the other in the uterus as the sole determining cause of the reaction of the uterus to adrenalin, it is necessary to imagine different mechanisms for the operation of parturition in the different types of animals, at all events if it is allowed that the glands of internal secretion, particularly the suprarenals, play any part. For example, if suprarenal secretion is evoked, perhaps by painful uterine contraction, in the first stage of parturition, the effect on the uterus of the rabbit would be diametrically opposite to the effect on the uterus of the guinea-pig. That such differences in the effect of an important activating agent can exist in two animals as alike as the rabbit and the guinea-pig would seem unlikely on general considerations—true as regards parturition there is one great point of difference in these animals; the young rabbit is born in a comparatively early stage of development, whilst the new-born guinea-pig has already reached a stage of development considerably more advanced, so that one might perhaps concede that whilst in the guinea-pig, where the size of the foetus is large compared with that of the mother, periodic intermissions in the contractions of the uterus may be necessary in order to allow time for the due expansion of the pelvic parts, separation of the pubic symphysis and so on, such intermissions are unnecessary in the rabbit, in which the relative size of the foetus at full term is very considerably smaller. On the other hand it must be borne in mind that all animals whose uterus is contracted by adrenalin do not normally bring forth their young at a correspondingly undeveloped stage: the human uterus for example behaves to adrenalin like the uterus of the rabbit.

In consequence, then, of the "abnormal" response referred to above and of the difficulty in postulating the preponderance of one type of nerve-ending over the other as the sole determining cause of the kind of reaction of the uterus to adrenalin, I have carried out the experiments recorded in this communication with the idea of showing that other factors may be involved.

These experiments were (*a*) on the isolated uterus, (*b*) on the uterus *in situ* in the intact animal, (*c*) feeding experiments followed by experiments on the uterus either isolated or *in situ* (sometimes the same uterus was used for experiment first in the intact animal and then as an excised organ).

### *Experiments on the isolated uterus.*

The animals were killed by pithing or by a blow on the head: the vessels in the neck were cut and the animal rapidly bled: the uterus was removed and washed in Ringer's solution, afterwards being placed in a dish of Ringer's solution which was repeatedly changed until the solution ceased to be coloured by blood from the uterine vessels: the uterus was left in Ringer's solution at laboratory temperature until transferred to the bath of warm Ringer-Locke solution for the actual experiment. For experiment, one horn of the isolated uterus, or sometimes a longitudinal strip of uterine horn in the case of the pregnant organ, was suspended in a bath of Ringer-Locke solution at 38° C. so that it pulled on a counterbalanced lever which recorded contractions by an upstroke of the writing-point. The Ringer-Locke solution had a composition of:

| | | |
|---|---|---|
| NaCl ... | 9·00 grams | in 1000 c.c. of glass-distilled water. |
| KCl ... ... | 0·42 „ | |
| $CaCl_2$ ... | 0·24 „ | |
| $NaHCO_3$ ... | 0·50 „ | |
| Dextrose ... | 1·00 „ | |
| Oxygen ... | saturated | |

The bath contained 80 c.c. of this solution which was kept oxygenated by a constant stream of oxygen bubbling through it. The apparatus used was a modification of that described by Roth (6), the chief modification being that the outer water-bath was of considerably greater capacity in comparison with the capacity of the contained bath of Ringer-Locke solution, so that any slight variation in the temperature of the water supplied to the water-bath would have less effect on the temperature of the solution in the contained bath. In practice it was found that the temperature of the water-bath could be maintained without difficulty at a level constant within the limits of 0·5° C. In accurate work on the isolated uterus these details are of importance, as this organ is very sensitive to slight changes in temperature and in oxygen saturation of the solution. After suspension of the uterus in the warm Ringer-Locke solution, sufficient time (usually 15–20 minutes) was allowed for the tonus to become steady, so that at the time when the experiments actually started the tonus of the uterus was slightly above that of extreme relaxation and the movements were small. If the experiment is started before this stage is reached it is difficult to be certain of the degree of tonus existing in the uterus and the individual movements may be inconveniently large. In some cases the drugs were added to the bath by means of a pipette in such a way that the drug was introduced away from the suspended organ and mixed with the solution by bubbling air through the bath: in other cases the drug already mixed with the Ringer-Locke solution to the required concentration was supplied from one of the containers within the water-bath.

In all some 130 experiments were performed: the organs were obtained from guinea-pigs, rats, cats and rabbits, and were in all stages of functional activity, virgin, non-pregnant, in different stages of pregnancy and taken at different periods after the termination of a pregnancy.

Fig. 1.

Isolated uterus of a guinea-pig (non-pregnant). Uterine movements (upstroke =contraction): Base-line and time-marking in seconds. At the arrow pituitrin was added to the Ringer-Locke solution to a concentration of 1–8000.

Isolated uterus of a guinea-pig (non-pregnant). Uterine movements (upstroke =contraction): Base-line and time-marking in 10 seconds. At the arrow adrenalin was added to the Ringer-Locke solution to a concentration of 1–1,000,000.

(In these and all other figures the tracings read from left to right.)

At first a few experiments were carried out applying one drug only, either adrenalin or pituitrin, to each uterus: in this way the capabilities of the apparatus were determined and a series of normal reactions to different concentrations of these drugs was obtained[1]. The usual effects of these drugs were obtained, viz. increased tonus and a series of gradually increasing movements starting in high tonus with pituitrin, and in the case of adrenalin relaxation of tonus and cessation of movements in the guinea-pig, rat and non-pregnant cat, increased tonus and movements in the case of the pregnant cat and rabbit. In the case of the guinea-pig, then, the effects of the two drugs are as opposed to each other as they can well be (Fig. 1).

---

[1] Various preparations were used of both adrenalin and pituitrin: all comparative results were obtained with the preparations of Messrs Parke Davis and Co. put up in bottles, not in ampoules: the contents of the latter are in my experience less reliable than the contents of stoppered bottles.

If these two drugs are applied together, and so long as their relative proportions in the mixture are approximately correct (it is impossible to generalize as to the proportions which balance each other, as even with recently "standardized" preparations the variations in activity are somewhat large, so that the proportions were apt to vary with different batches of the drugs: further there appears to be considerable variation in sensitiveness to one or other drug between individual organs), the effect obtained is a very definite combination of the effects of the drugs when given separately: the uterus shows regular large movements the extent of which does not fall far short of that between complete relaxation and extreme contraction (Fig. 2). If the proportion of pituitrin in the mixture is unduly large the effect partakes more of the nature of a pure pituitrin response, that is to say the tonus is increased and increased movements are observed in a state of high tonus, but the

Fig. 2. Isolated uterus of a guinea-pig (non-pregnant). Uterine movements (upstroke = contraction): Base-line and time-marking in seconds. At the arrow a mixture of adrenalin and pituitrin was added to the Ringer-Locke solution to concentrations of 1–500,000 and 1–10,000 respectively.

relaxations are more distinct than in the case of a pure pituitrin response (Fig. 3).

If the uterus is treated with adrenalin for some time before suspension and pituitrin is added subsequently, the effect is either the ordinary pure pituitrin effect or such an effect as is produced by a mixture of the two drugs. But if the uterus is first treated with pituitrin and then after suspension adrenalin is applied, one obtains, not the ordinary adrenalin effect of relaxation and cessation of movements, nor does one obtain an effect similar to that produced by a mixture of the two drugs, but an effect comparable with that produced by applying pituitrin, that is to say that under these conditions the effect of adrenalin is reversed (Fig. 4).

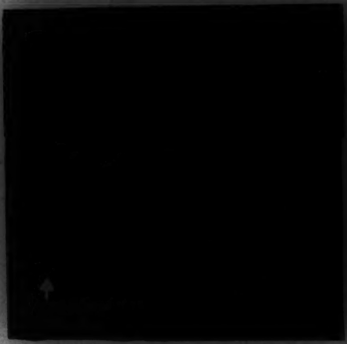


Fig. 3. Isolated uterus of a guinea-pig (non-pregnant). Uterine movements (upstroke = contraction): Base-line and time-marking in seconds. At the arrow a mixture of adrenalin and pituitrin was added to the Ringer-Locke solution to concentrations of 1–1,000,000 and 1–10,000 respectively.

Fig. 4. Isolated uterus of a guinea-pig (non-pregnant). Uterine movements (upstroke = contraction): Base-line and time-marking in seconds. At the arrow adrenalin was added to the Ringer-Locke solution to a concentration of 1–1,000,000. This uterus had previously been left in a solution of pituitrin (1–10,000) for 60 minutes.

Since the uterus of the guinea-pig usually reacts to adrenalin in the same way whether pregnant or not—always by relaxation—one would expect that this reversed response to adrenalin in the uterus previously "sensitized[1]" by pituitrin would be obtained both in the pregnant and non-pregnant organ of this species, and this was found to be the case. It appeared too that in the case of the pregnant uterus of this animal the reversed response to adrenalin was easier to obtain and with less sensitizing than in the case of the non-pregnant uterus.

---

[1] In a communication published in 1915 **Blair Bell** (7) describes his procedure of "sensitizing" the uterus with pituitrin during the last two or three months of pregnancy in cases of suspected idiopathic uterine inertia. Though he appears to have had no definite experimental data to guide him and though he advances no proof that this

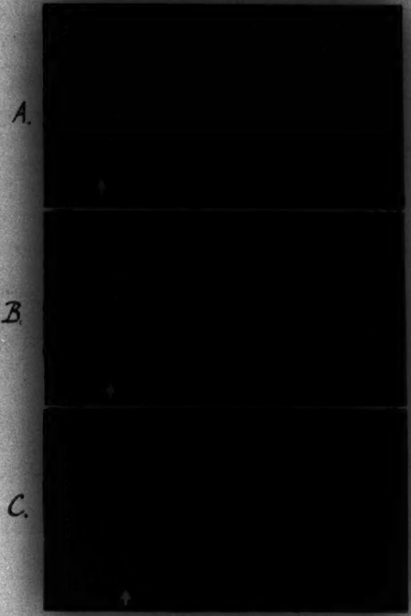


Fig. 5. Isolated uterus of a rat (pregnant about 10 days). Uterine movements (upstroke =contraction): Base-line. At the arrows adrenalin was added to the Ringer-Locke solution to a concentration of 1–500,000. A=normal uterine horn. B=the same horn previously "sensitized" in pituitrin (1–5000) for 45 minutes. C=the same horn further "sensitized" in pituitrin (1–5000) for a further 30 minutes.

Similarly, preliminary sensitizing with pituitrin produced a contraction response to adrenalin in the virgin or non-pregnant uterus of the cat: in the pregnant uterus of this animal no change was observed, as was to be expected, since when pregnant the cat's uterus normally responds to adrenalin by contraction.

"sensitizing" takes place, the rationale of his treatment seems to be identical with what I am describing: consequently I have thought well to retain the term "sensitize."

Again in the case of the rabbit's uterus, which normally responds to adrenalin by contraction whatever its functional state may be, no change was observed as a result of preliminary sensitizing with pituitrin.

Though the uterus of the rat responds to adrenalin in the same way as that of the guinea-pig—by relaxation in all states of functional activity—a difference was noticed between the organs of the two species in connection with this reversed response to adrenalin: the guinea-pig's uterus, as has been shown, can readily be made to respond to adrenalin by contraction: the uterus of the rat on the other hand is less amenable to this treatment; only with the greatest difficulty can the sensitizing process be carried to the necessary extent; the usual effect of progressive sensitizing, even in the pregnant organ of this animal, is that the degree of inhibition produced by subsequent application of adrenalin becomes less and less marked as regards both duration and degree as the process of sensitizing lengthens (Fig. 5).

A few experiments were carried out with the object of determining whether the presence of products of conception within a horn of pregnant uterus made any difference in the response. No difference was noticed in any case, a longitudinal strip of the horn of a pregnant uterus responding in exactly the same way to adrenalin and to pituitrin as did the intact pregnant horn. Again in one or two instances records were taken of the movements of a pregnant horn intact and later of the same horn with the contents removed. Confirmation was obtained in yet another way: on one or two occasions a bicornuate uterus was found to be pregnant in one horn and non-pregnant in the other: each horn was found to react to both adrenalin and pituitrin in exactly the same way.

On a few occasions rings of circular muscle were taken from the uterus of a guinea-pig, cut through and suspended as strips of circular muscle. It was found in certain instances, particularly in rings cut from the vaginal end of the uterus, that pituitrin produced a definite relaxation.

### *Experiments on the uterus* in situ *in the intact animal.*

Non-pregnant female cats were the animals used: they were kept under observation in the laboratory so that their reproductive history was known for at least one month before experiment: some were fed with pituitary preparations and received subcutaneous injections of pituitrin during this period; others received no such treatment. For experiment, the animals were anæsthetized with A.C.E. mixture and then with urethane (in a few instances the animals were decerebrated instead); a respiration tube was tied into the trachea; a cannula was tied into the jugular vein; the carotid blood-pressure was recorded by a mercury manometer; one hypogastric nerve was isolated and placed on suitable electrodes; the uterine movements were recorded by placing a thread, connected over pulleys with a counterbalanced lever, beneath both horns of the uterus, which remained intact and *in situ*. The edges of the abdominal wound were propped up with small glass supports, so that the wound formed the rim of a deep cup formed by the abdominal cavity, which was filled with warm Ringer's solution completely immersing the uterus. Those animals which had received no preliminary treatment with pituitrin received first an intravenous injection of adrenalin which produced the normal effects both on blood-pressure and on the uterine movements. Then injections of pituitrin were given slowly and at short intervals over a period of 15–30 minutes, after which a second injection of adrenalin was given, when it was found that the uterus responded by increase in tonus and movements instead of by relaxation. An exactly similar change was noticed as the result of stimulating the hypogastric nerve before and after the injection of pituitrin. Some of the animals which had received preliminary treatment with pituitary preparations gave the reversed uterine response to hypogastric stimulation or to injection of adrenalin without any further injection of pituitrin (Fig. 6).

Fig. 6. Cat (non-pregnant): urethane. Uterine movements (upstroke = contraction): Base-line and time-marking in seconds. At the arrow 0·5 c.c. adrenalin (1–10,000) was injected intravenously. The animal had previously received daily doses of pituitary preparations by the mouth and subcutaneous injections of pituitrin over a period of six weeks. The animal was known not to have been pregnant for five months. (In this exp. the blood-pressure tracing continually obliterated the tracing of uterine movements, so the former was discontinued.)

### *Feeding experiments.*

Guinea-pigs and cats—all females and non-pregnant—were used. The cats received with their milk a daily dose of desiccated posterior lobe of pituitary body (Armour), starting with 0·05 gm. each daily, gradually increasing up to 0·5 gm. daily over a period of 5–7 weeks. During this period too they received subcutaneous injections of pituitrin, starting with 0·1 c.c. increasing to 0·4 c.c. or 0·5 c.c. Each animal received from 20 to 25 such injections during this period, the injection being omitted one day in every six or seven. The guinea-pigs received similar treatment varying in duration from 30 days to 18 weeks: the injections of pituitrin started with 0·05 c.c. for a dose, gradually increased up to 0·15 c.c., 0·2 c.c., 0·25 c.c., or 0·3 c.c. These animals too received by the mouth Armour's desiccated posterior lobe of pituitary body or Duncan and Flockhart's pulv. ext. pituitary in daily doses gradually increasing from 0·05 gm. to 0·3 gm.

The animals were then used for experiment, the guinea-pigs being killed and the uterus removed and treated as an isolated organ; the cats in some instances being anæsthetized and a record being taken of the movements of the intact uterus, and in other cases the animals being killed outright and the uterus removed and suspended as an isolated organ.

It was found in both instances that this form of preliminary treatment with pituitary preparations was sufficient to sensitize the uterus so that subsequent application of adrenalin, either intravenously in the case of the intact cat or into the Ringer-Locke solution in which the uterus was suspended in the case of the isolated organ, would produce the reversed uterine response to adrenalin: similarly in the case of the intact cat hypogastric nerve stimulation would evoke contraction instead of relaxation of the uterus.

It would appear from the foregoing results that an unwontedly generous supply to the uterus of the active principle of the posterior lobe of the pituitary body is sufficient stimulus to change in some way the reaction to adrenalin of the uterus of such species of animals as normally reacts to adrenalin (or to hypogastric nerve stimulation) by relaxation. It is known that in the case of the cat the uterus normally and without adventitious aid changes its relaxation response for a contraction response to adrenalin during pregnancy. It has further been shown that the pregnant uterus of the guinea-pig is more easily sensitized by pituitrin for this contraction response to adrenalin than is the non-pregnant uterus of this animal, and further that the uterus of the guinea-pig is more easily sensitized than is the uterus of the rat, irrespective of the functional state of the organ. One can thus bring into line all species of animals; with the rabbit, dog, ferret, monkey and man at one end of the series, where the uterus is normally contracted by adrenalin in both pregnant and non-pregnant states; with the cat occupying an intermediate position; and with the guinea-pig and rat at the other end of the series. Further one can see that all that is necessary so to bring into line all types of uterus is a supply of the active principle of the posterior lobe of the pituitary body, a smaller supply in the case of the rabbit and a larger supply in the case of the guinea-pig and rat. What may be the exact significance of this variation in the amount of pituitary active principle which is necessary it is difficult to say.

It is recognized from the work of Erdheim and Stumme(8) and from the observations of Marek(9), Blair Bell(10) and others that during pregnancy the pituitary body undergoes a definite hyperplasia with the appearance of specialized "pregnancy cells" and the setting up of a degree of activity greater than is found in the non-pregnant animal. There seems, too, little reason to doubt that the pars intermedia and pars nervosa share in this increased activity, so that there is in the natural economy a provision whereby an increased supply of pituitrin is available during pregnancy.

There is then no necessity for the hypothesis, that during pregnancy the motor nerve-endings in the uterus multiply to such an extent that they come to outnumber the inhibitor nerve-endings, since we have all the factors necessary for this change in response without having to fall back on any such hypothesis.

An attempt was made to place this sensitizing action of pituitrin: in the first place a systematic series of experiments was undertaken on the isolated uterus of the guinea-pig, using other drugs instead of adrenalin and comparing the effects produced by these drugs before and after sensitizing with pituitrin. The drugs used were arecoline, pilocarpine, pituitrin, liquid extract of ergot, ergotoxine, tyramine and barium. It was found that preliminary treatment with pituitrin sensitizes the uterus for the subsequent action of pituitrin, arecoline, pilocarpine, ergot, ergotoxine and tyramine, but not for that of barium. That is to say after preliminary treatment with pituitrin subsequent application of these other drugs produces an effect, when in concentration lower than that necessary to produce an effect on the normal uterus: similarly the same concentration will produce a more noteworthy result on the sensitized than on the normal uterus, so long as the concentration is such that a submaximal response is obtained.

It has been seen that this sensitizing action of pituitrin applies to adrenalin, a drug known to act by stimulation of sympathetic myoneural junctions: the action applies also to ergotoxine and tyramine, drugs again which produce their actions through the peripheral sympathetic system: again with arecoline and pilocarpine, drugs which stimulate the peripheral autonomic system, either sympathetic or cranio-sacral autonomic, the same holds good. With barium on the other hand, a drug which produces its action by a direct stimulation of the muscle, no such effect was produced.

It would seem, then, that the seat of this sensitizing action of pituitrin lies not in the muscle itself but in some part of the peripheral innervation: if there is no cranio-sacral autonomic innervation in the uterus, as some authorities hold, then this action of pituitrin would appear to lie in the peripheral sympathetic innervation: if there is also a cranio-sacral autonomic innervation in the uterus, then this sensitizing action would appear to lie in the peripheral parts of either sympathetic or cranio-sacral autonomic or both. The secondary application of pituitrin to the sensitized uterus throws little or no light on this point, since the seat of action of pituitrin itself is placed by different observers in different localities, thus Dale(11) places it in the end-organ independently of its innervation, or at any rate in some point nearer the periphery than the point on which adrenalin acts, whilst others consider that pituitrin stimulates autonomic nerve-endings or some particular innervation(12).

Kepinow(13) states that a preliminary injection of pituitrin affects the vasomotor mechanism of rabbits in such a way that the effect of a subsequent injection of adrenalin is exaggerated: he found too a similar augmented effect on the pupil of cats and rabbits. I repeated these experiments on the blood-pressure of cats, and found ample confirmation of Kepinow's statement. In one experiment, whereas before the application of pituitrin an intravenous injection of adrenalin (0·025 mg.) produced a rise in carotid blood-pressure from 71 to 158 mm. Hg, a similar injection after the animal had received 5 c.c. of 2·5 per cent. pituitrin, injected intravenously in 34 separate injections over a period of 25 minutes, produced a rise from 40 to 248 mm. Hg.

If this effect of pituitrin on the blood-pressure is the same as that already described on the uterus, it would appear to be an effect on either the sympathetic or the musculature. In another experiment

1 c.c. of a 0·5 per cent. solution of barium chloride was injected intravenously before and after 5 c.c. of 2·5 per cent. pituitrin given by slow injection lasting over a period of 30 minutes. In this case the rise in blood-pressure resulting from the injections of barium was identical—22 mm. Hg—in each case.

It appears then that on two separate body mechanisms, each consisting essentially of a sympathetic innervation and a plain muscle end-organ, pituitrin produces a sensitizing effect for the subsequent action of adrenalin, but that whilst this sensitizing applies to drugs which act through the sympathetic it does not apply to drugs which directly stimulate the plain muscle.

A few experiments were then carried out both on the isolated uterus and on the blood-pressure of the intact animal with the object of determining whether or not this sensitizing process would take place on the mechanism previously treated with drugs known to paralyze peripheral nervous structures whilst leaving the muscle intact. It was found in both instances with ergotoxine and apocodeine that no such sensitizing occurred, though the musculature was shown to be normally active (or approximately so) to barium.

One is forced then to the conclusion that this sensitizing action of pituitrin, whatever be its real nature, is an action on some part of the peripheral mechanism central to the end-organ.

Fröhlich and Pick(14) have published the statement that extracts of hypophysis remove the paralysis of sympathetic vaso-constrictor nerve-endings produced by ergotoxine—in other words that pituitrin annuls Dale's vasomotor paradox. It appeared that this effect and the sensitizing action of pituitrin which I have described might have a common foundation. In three separate attempts on cats I have failed to obtain the effect which Fröhlich and Pick describe. Dale in a personal communication tells me that he too has failed to obtain the effect described by Fröhlich and Pick. Is it possible that in Fröhlich and Pick's experiments sufficient pituitrin remained in the cannula and vein to produce the effect which they ascribe to adrenalin? It is known that the pressor effect of pituitrin is unaffected by ergotoxine paralysis.

Part of the work on which this communication is founded was carried out during the tenure of a Beit Memorial Research Fellowship.

Part of the expenses of this research was defrayed by a grant from the Government Grant Committee of the Royal Society.

## SUMMARY AND CONCLUSIONS.

1. Experiments are described in which adrenalin and pituitrin in combination and following each other were applied to the uterus.

2. Preliminary treatment of the uterus with pituitrin produces a reversal of the normal adrenalin response of the uterus of the guinea-pig and of the virgin or non-pregnant cat.

3. The presence or absence of products of conception in the pregnant uterus makes no difference to the response of the uterus to either adrenalin or pituitrin.

4. Pituitrin under certain conditions may produce a relaxation of the circular muscle fibres of the uterus.

5. The suggestion is made that a determining factor of the response of the uterus to adrenalin is the amount of active principle of the posterior lobe of the pituitary body which has been available.

6. This "sensitizing" action of pituitrin appears to reside in the peripheral nervous mechanism probably of the sympathetic system: it does not reside in the end-organ.

7. A parallelism is drawn between this "sensitizing" action of pituitrin on the uterus and an, at first sight rather different, effect which it produces on the vasomotor mechanism.

8. The statement of Fröhlich and Pick that extracts of hypophysis remove the paralysis of sympathetic vaso-constrictor nerve-endings produced by ergotoxine is not confirmed.

## REFERENCES.

(1) **Dale.** This Journal, 34. 163. 1906.
(2) **Langley and Anderson.** Ibid. 19. 122. 1895.
    **Langley.** Ibid. 27. 252. 1901.
(3) **Cushny.** Ibid. 35. 1. 1906.
    **Dale.** Ibid. 34. 163. 1906.
    **Kehrer.** Archiv f. Gynäk. p. 169. 1906.
(4) **Gunn and Gunn.** Journ. of Pharm. and exp. Ther. 5. 527. 1914.
(5) **Cow.** This Journal, 48. 443. 1914.
(6) **Roth.** Journ. of Pharm. and exp. Ther. 5. 563. 1914.
(7) **Blair Bell.** Proc. Roy. Soc. Med. 8. 1915. (Sect. Obstet. p. 71.)
(8) **Erdheim and Stumme.** Berl. klin. Woch. 45. 1031. 1908. Ziegler's Beitr. 46. 1. 1909.
(9) **Marek.** Zntrlb. f. Gynäk. 35. 1612. 1911.
(10) **Blair Bell.** Sex Complex, p. 55. London, 1916.
(11) **Dale.** Biochem. Journ. 4. 427. 1909.
(12) **v. Frankl-Hochwart and Fröhlich.** Arch. f. exp. Path. u. Pharm. 63. 347. 1910.
    **Fröhlich and Pick.** Ibid. 74. 92. 1913.
(13) **Kepinow.** Ibid. 67. 247. 1912.
(14) **Fröhlich and Pick.** Ibid. 74. 114. 1913.

# SOME FURTHER OBSERVATIONS ON BLOOD REACTION. BY J. F. DONEGAN AND T. R. PARSONS.

*(From the Physiological Laboratory, Cambridge.)*

CONTENTS.

PAGE

I. The range of variation in the reaction of the blood . . 319

II. The relation between the reaction and the carbon dioxide combining power of blood . . . . . . 322

III. The relation between the reaction and the oxygen-combining power of blood . . . . . . 324

DURING the past year we have been engaged on the examination of the blood of some of the patients—mostly soldiers—who have been sent for "oxygen treatment" to Cambridge. Our experiments on each blood have usually consisted in the determination at various $CO_2$-tensions of the hydrogen-ion concentration, the total $CO_2$-content and the value of $\log K$ in Hill's well-known equation to the oxygen dissociation curve of blood(1), and we have thought it desirable to collect together all these measurements not so much with the object of demonstrating any particular effect of the long-continued inhalation of oxygen, but in order to extract from them such information as they yield with regard to the relation between the reaction of blood in general and its $CO_2$- and oxygen-combining power. We have been stimulated to this by the pioneer work of Hasselbalch on this subject(2).

While the subjects from whom we have received samples of blood have not been, unfortunately, in a normal state of health, we have been careful to exclude from our list observations on cases (*e.g.* uræmias) in which it is known that a marked change of the properties of the blood occurs—these are to be dealt with in another place.

In addition to these measurements we have a few more observations to record on the (presumably) normal blood of Parsons together with some experiments on the effect of small quantities of lactic acid on the properties of that blood.

The apparatus and methods employed in this research form part of those described in a former paper by one of us (3) to which a reference will suffice. In the $p_H$ measurements we were able to work only on the completely reduced blood so that we had no need of the centrifuging apparatus, but could pass the blood direct from saturator to electrode vessel. As before we have been careful always to use thoroughly cleaned and newly platinised platinum electrodes and a frequently standardised calomel electrode throughout. The measurement of the total $CO_2$-contents of the samples of reduced blood was carried out in a Barcroft's differential blood-gas apparatus calibrated in the manner already described, and, finally, the values for log $K$ were obtained by measuring the percentage saturation of the blood by means of the same apparatus after it had been brought into equilibrium with an analysed gas mixture containing nitrogen, oxygen, and carbon dioxide at suitable tensions. As such a complete investigation requires at least two days, it was unavoidable that the blood sample should have to be preserved on ice overnight, but we have performed the log $K$ and $p_H$ determinations alternately, so that any change which may have occurred in the blood on standing has affected both these values together and so has not vitiated their comparison. In the experiments on the effect of lactic acid, this substance was used in concentrated solution (B.P. sp. gr. 1·21, *i.e.* about 75 p.c.), the small measured volume of it being allowed to diffuse slowly into the blood from the point of a capillary pipette—a procedure which produced no visible change in the blood such as precipitation of proteins or damage to the red cells. Naturally we have only an approximate estimate of the actual quantity of lactic acid present in our mixtures, but this is all we require for our present purpose.

A full list of the results of our experiments is given in Table I, and for the sake of adequate representation we have plotted the values in the series of curves shown in Fig. 1. The series of curves on the left of each diagram represent the properties of the sample of blood received before the subject was treated with oxygen; those on the right refer to the sample received after the treatment, *i.e.* some 5 or 6 days later than the first. In the case of Parsons' blood, in addition to the curves obtained before and after a period of oxygen inhalation in July 1918, we show for comparison the $p_H$ and $V_{CO_2}$ curves obtained for the reduced blood in September and October 1917 (represented by means of broken lines in the figure) and also the results of three $p_H$ measurements made in February 1918 (represented as circles). These curves are of importance as indicating the extent of the change which appears to have been taking

TABLE I.

| Blood | Tension of $CO_2$ in mm. Hg. $P_{CO_2}$ | $p_H$ | Total $CO_2$ content c.c./100 c.c. $V_{CO_2}$ | Tension of $CO_2$ in mm. Hg. $P_{CO_2}$ | Log $K$ |
|---|---|---|---|---|---|
| G.<br>Before treatment | ·7 | 8·24 | 4·6 | ·3 | $\bar{3}$·43 |
| | 9·7 | 7·73 | 33·8 | 9·8 | $\bar{4}$·90 |
| | 29·6 | 7·46 | 46·3 | 25·2 | $\bar{4}$·53 |
| | 44·0 | 7·34 | 56·1 | 36·6 | $\bar{4}$·41 |
| | 65·7 | 7·24 | 65·2 | 52·8 | $\bar{4}$·24 |
| | — | — | — | 69·4 | $\bar{4}$·26 (?) |
| After treatment | 1·3 | 8·21 | 6·5 | ·4 | $\bar{3}$·35 |
| | 12·2 | 7·70 | 31·3 | 9·5 | $\bar{4}$·85 |
| | 37·0 | 7·40 | 47·5 | 25·6 | $\bar{4}$·53 |
| | 67·6 | 7·19 | 57·1 | 41·5 | $\bar{4}$·32 |
| | — | — | — | 50·1 | $\bar{4}$·25 |
| | — | — | — | 77·9 | $\bar{4}$·10 |
| H.<br>Before treatment | 2·1 | 8·21 | 8·5 | ·6 | $\bar{3}$·29 |
| | 18·8 | 7·62 | 37·8 | 10·1 | $\bar{4}$·84 |
| | 31·5 | 7·44 | 50·2 | 24·3 | $\bar{4}$·45 |
| | 44·2 | 7·33 | 56·5 | 37·6 | $\bar{4}$·28 |
| | — | — | — | 54·4 | $\bar{4}$·26 (?) |
| After treatment | 2·2 | 8·25 | 10·5 | ·3 | $\bar{3}$·31 |
| | 18·1 | 7·62 | 40·9 | 11·7 | $\bar{4}$·70 |
| | 38·9 | 7·39 | 46·5 | 27·3 | $\bar{4}$·37 |
| | 59·3 | 7·25 | 58·0 | 39·6 | $\bar{4}$·24 |
| | — | — | — | 40·0 | $\bar{4}$·30 |
| | — | — | — | 47·7 | $\bar{4}$·23 |
| | — | — | — | 54·0 | $\bar{4}$·21 |
| L.<br>Before treatment | — | — | — | ·4 | $\bar{3}$·30 |
| | — | — | — | 10·3 | $\bar{4}$·76 |
| | 21·8 | 7·56 | — | 25·4 | $\bar{4}$·47 |
| | — | — | — | 36·4 | $\bar{4}$·36 |
| | 44·5 | 7·33 | — | 46·9 | $\bar{4}$·23 |
| | 59·5 | 7·24 | — | 64·9 | $\bar{4}$·12 |
| After treatment | 1·3 | 8·18 | 7·8 | ·8 | $\bar{3}$·47 |
| | 20·0 | 7·58 | 36·9 | 12·5 | $\bar{4}$·80 |
| | 42·7 | 7·35 | 48·0 | 28·7 | $\bar{4}$·48 |
| | 65·9 | 7·23 | 57·5 | 38·6 | $\bar{4}$·41 |
| | — | — | — | 52·3 | $\bar{4}$·24 |
| S.<br>Before treatment | 1·3 | 8·26 | 10·1 | ·6 | $\bar{3}$·48 |
| | 15·9 | 7·65 | 36·6 | 10·9 | $\bar{4}$·81 |
| | 38·2 | 7·40 | 49·5 | 25·3 | $\bar{4}$·48 |
| | 51·5 | 7·295 | 54·2 | 38·7 | $\bar{4}$·35 |
| | 59·3 | 7·25 | — | 51·6 | $\bar{4}$·23 |
| | — | — | — | 68·4 | $\bar{4}$·10 |

TABLE I (*continued*).

| Blood | Tension of CO$_2$ in mm. Hg. $P_{CO_2}$ | $p_H$ | Total CO$_2$ content c.c./100 c.c. $V_{CO_2}$ | Tension of CO$_2$ in mm. Hg. $P_{CO_2}$ | Log $K$ | |
|---|---|---|---|---|---|---|
| S. After treatment | 1·0 | 8·27 | 12·5 | ·5 | $\bar{3}$·33 | |
| | 21·3 | 7·57 | 40·0 | 12·2 | $\bar{4}$·69 | |
| | 40·5 | 7·36 | 49·7 | 40·9 | $\bar{4}$·27 | |
| | 60·0 | 7·25 | 56·1 | 57·9 | $\bar{4}$·15 | |
| T. Before treatment | 2·0 | 8·14 | 10·3 | 1·5 | $\bar{3}$·15 | |
| | 14·2 | 7·66 | 30·3 | 11·3 | $\bar{4}$·63 | |
| | 30·8 | 7·42 | 41·0 | 25·9 | $\bar{4}$·36 | |
| | 45·9 | 7·33 | 48·2 | 39·4 | $\bar{4}$·23 | |
| | 60·3 | 7·24 | — | 53·9 | $\bar{4}$·15 | |
| After treatment | 2·0 | 8·22 | 8·6 | 1·0 | $\bar{3}$·13 | |
| | 19·1 | 7·60 | 34·8 | 10·1 | $\bar{4}$·66 | |
| | 40·8 | 7·38 | 45·4 | 41·7 | $\bar{4}$·21 | |
| | 60·7 | 7·26 | 59·0 | 54·0 | $\bar{4}$·10 | |
| Parsons, Feb. 1918 | 11·0 | 7·77 | — | — | — | |
| | 31·6 | 7·48 | — | — | — | |
| | 60·7 | 7·26 | — | — | — | |
| Before treatment (July 1918) | 10·7 | 7·77 | — | ·3 | $\bar{3}$·07* | $\bar{3}$·08* |
| | 31·6 | 7·43 | — | 17·4 | $\bar{4}$·43 | $\bar{4}$·41 |
| | 44·8 | 7·33 | — | 32·9 | $\bar{4}$·28 | $\bar{4}$·27 |
| | 52·9 | 7·29 | — | 42·2 | $\bar{4}$·22 | $\bar{4}$·22 |
| | — | — | — | 57·6 | $\bar{4}$·07 | — |
| | — | — | — | 57·5 | — | $\bar{4}$·04 |
| After treatment (July 1918) | 10·9 | 7·73 | 26·7 | ·9 | $\bar{3}$·07* | $\bar{3}$·03* |
| | 29·3 | 7·46 | 39·8 | 16·1 | $\bar{4}$·54 | $\bar{4}$·45 |
| | 45·3 | 7·31 | 49·3 | 29·6 | $\bar{4}$·25 | $\bar{4}$·32 |
| | 59·0 | 7·22 | — | 43·4 | $\bar{4}$·16 | $\bar{4}$·16 |
| | — | — | — | 59·0 | $\bar{4}$·08 | — |

* Duplicate determinations.

| | | | | | |
|---|---|---|---|---|---|
| Parsons + about ·04 p.c. lactic acid | ·7 | 8·01 | 8·9 | — | — |
| | 11·6 | 7·57 | 28·2 | — | — |
| | 31·3 | 7·36 | 39·1 | — | — |
| | 46·1 | 7·26 | 44·5 | — | — |
| | 62·3 | 7·16 | — | — | — |
| + about ·05 p.c. lactic acid | 1·7 | 7·94 | — | ·2 | $\bar{3}$·03 |
| | 12·5 | 7·60 | 23·8 | 9·9 | $\bar{4}$·57 |
| | 25·6 | 7·41 | 32·1 | 25·3 | $\bar{4}$·27 |
| | 40·9 | 7·27 | 39·7 | 38·9 | 4·12 |
| | 55·1 | 7·19 | — | 53·9 | $\bar{4}$·07 |
| | — | — | — | 53·9 | $\bar{4}$·07 |
| | — | — | — | 66·8 | $\bar{5}$·94 |

place in Parsons' blood since the time of the first investigation. In many cases our experiments have been somewhat less complete than we would have wished, but from the results here shown we shall venture to draw certain general conclusions.

### I. *The range of variation in the reaction of the blood.*

In the first place it is interesting to compare the reactions of these various samples of blood under the same conditions, and so to obtain an estimate of the degree of individual variation among them. For example, from the $p_H/P_{CO_2}$ curves in Fig. 1 we have read off the $p_H$ of each specimen at 40 mm. $CO_2$-tension, *i.e.* the value which Hasselbalch has termed the "reduced $p_H$" (4) for each blood. When these values, which are recorded on the diagrams, are compared among themselves it is seen that they all lie between the narrow limits of 7·36 and 7·38, with an average value of almost exactly 7·37, and seeing that the total range of variation is just equal to the experimental error on a single $p_H$ determination we may summarise all the results by stating that the reduced $p_H$ of each of the bloods examined was 7·37 ± ·01.

That this may express the condition of the blood of an individual in a state of normal health is shown by the determinations carried out on the blood of Parsons where, on two successive occasions in July 1918, the values for the reduced $p_H$ were 7·37 and 7·36 respectively. Now, as has already been mentioned, Parsons' blood was investigated fully on a previous occasion, and it was then found to be markedly more alkaline, the value of its reduced $p_H$ being 7·41 (3). At first it was thought that this difference in the results might be an apparent one only and due to some change in the apparatus used for the $p_H$ determination, but a careful calibration showed that this was not the case, that the apparatus had remained unchanged so that therefore this difference was the expression of a real change in the blood. It will be observed that this change had begun in Feb. 1918 when the reduced $p_H$ was found to have a value of 7·40 (see the diagram in Fig. 1).

It has been established by Haldane and his co-workers (5) that the rate of increase of lung ventilation with increasing $CO_2$-tension in the alveolar air is at the rate of about 10 litres per min. for a rise of tension of about 2 mm., and this rise of tension corresponds to a diminution in the $p_H$ of blood of about ·02. Therefore, if the hydrogen ions of the blood constitute the adequate stimulus to the respiratory centre, it is evident that during these changes in Parsons' blood some correspondingly marked adjustment must have been brought about either in the

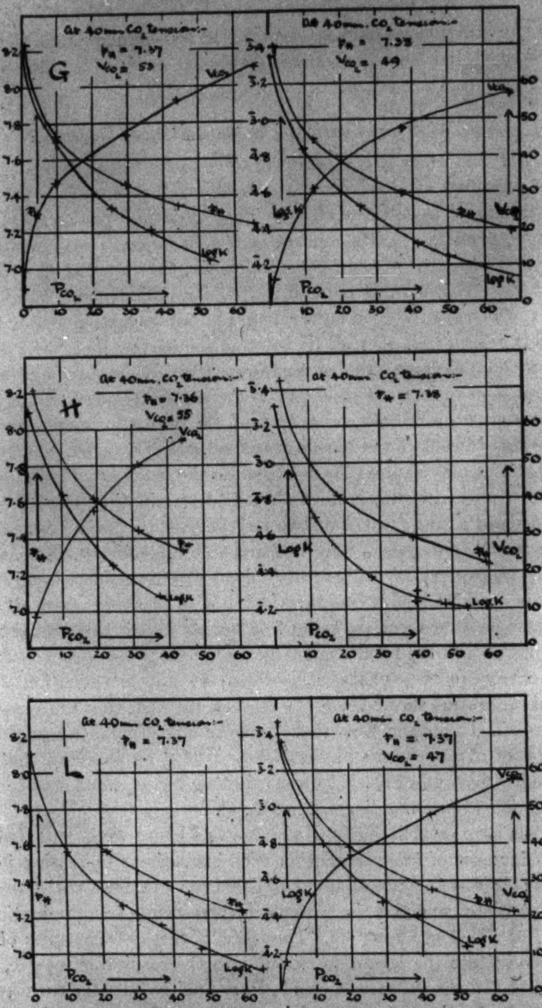


Fig. 1. For full explanation see text. In each case each of the three vertical scales is common to both halves of the diagram.

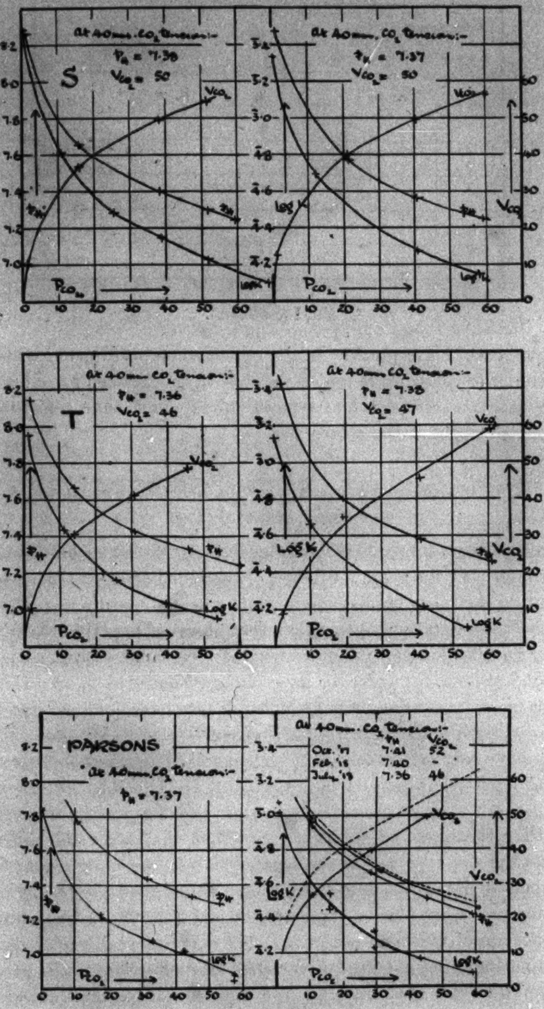


Fig. 1 (*continued*).

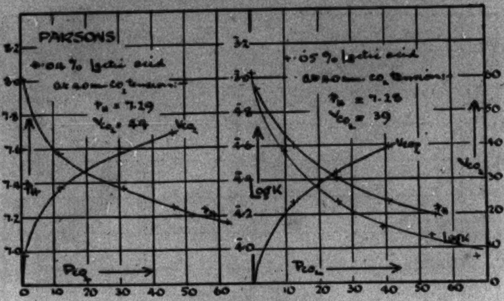


Fig. 1 (*continued*).

sensitiveness of his respiratory centre or in the composition of his alveolar air, in order to compensate for the decrease in the reduced $p_H$ of his blood to the extent of ·05; for his respiratory responses have remained unchanged throughout the whole period.

## II. *The relation between the reaction and the carbon dioxide combining power of blood.*

In connection with the first published data for Parsons' blood (3) it was pointed out that the value for the $p_H$ of the blood at any $CO_2$-tension by calculation from the $CO_2$-dissociation curve, according to Hasselbalch's method (2), was invariably lower than that observed experimentally. This observation has led us to enquire whether the same is true of those of our blood samples for which we have obtained both the $p_H$ and the $CO_2$-dissociation curves. For the sake of brevity we have confined our attention to one particular value of $CO_2$-tension—40 mm. Hg—and at that tension have calculated the $p_H$'s of the several bloods and compared the calculated with the observed values in Table II. It will be seen that here also we obtain a series of calculated values which are without exception lower than the observed values, and, further, that while the difference between the observed and the calculated $p_H$'s in the more recent determinations on Parsons' blood still remains unaltered at ·07, the differences for the other bloods vary between ·00 and ·09—a total range of difference about five times as great as the experimental error in our $p_H$ determinations, and a degree of variation of such extent as to preclude the possibility of a constant error in our method for estimating the total $CO_2$-contents of the bloods.

TABLE II.

Calculation of the $p_H$ from the $CO_2$-dissociation curve (Hasselbalch).

| Blood | Total $CO_2$ content at 40 mm. CO tension (c.c./100 c.c.) | $p_H$ at 40 mm. $CO_2$ tension |  | Difference between calculated and observed values |
|---|---|---|---|---|
|  |  | calculated | observed |  |
| G. |  |  |  |  |
| Before treatment | 53 | 7·34 | 7·37 | – ·03 |
| After treatment | 49 | 7·31 | 7·38 | – ·07 |
| H. |  |  |  |  |
| Before treatment | 55 | 7·36 | 7·36 | – ·00 |
| L. |  |  |  |  |
| After treatment | 47 | 7·29 | 7·37 | – ·08 |
| S. |  |  |  |  |
| Before treatment | 50 | 7·32 | 7·38 | – ·06 |
|  | 50 | 7·32 | 7·37 | – ·05 |
| T. |  |  |  |  |
| Before treatment | 46 | 7·29 | 7·36 | – ·07 |
| After treatment | 47 | 7·29 | 7·38 | – ·09 |
| Parsons |  |  |  |  |
| Oct. 1917 | 52 | 7·34 | 7·41 | – ·07 |
| July 1918 | 46 | 7·29 | 7·36 | – ·07 |
| Parsons + lactic acid |  |  |  |  |
| ·04 p.c. | 44 | 7·27 | 7·29 | – ·02 |
| ·05 p.c. | 39 | 7·22 | 7·28 | – ·06 |

There are several ways in which one might attempt to explain these results. In the first place it seems possible that the close agreement which Hasselbalch found to obtain between his calculated and observed results was due to some extent to his experimental procedure: that the calculated values of the $p_H$'s might be expected to be lower than those determined by experiment may be made more evident by the following considerations. There is no doubt of the applicability of Hasselbalch's calculations to homogeneous systems containing $CO_2$ and sodium bicarbonate in equilibrium. But while aqueous solutions of sodium bicarbonate and also blood plasma represent such systems, the same is not true of the whole blood. Here we have a two-phase system composed of corpuscles and plasma in which the differences of composition of the two phases lead to marked differences in their combining powers for $CO_2$. Therefore the relation between $CO_2$-tension and $CO_2$-content in the whole blood will be different from that in the plasma. Now it has been shown previously (3) that it is the relation between free and combined $CO_2$ in the *plasma* which determines the value obtained in an electrometric determination of the $p_H$ of the whole blood, while it is from the *different* relation between free and combined $CO_2$ in the *whole* *blood* that we obtain the calculated value of its $p_H$. In other words the difference between the observed and the calculated $p_H$'s of blood is simply an expression of the difference in behaviour of corpuscles and plasma towards $CO_2$. The question is an intricate one, and doubtless its final solution will involve a consideration of several other factors: for example, the relationship of Buckmaster's recent results (6) on the carriage of $CO_2$ by hæmoglobin has yet to be made out in this connection.

### III. *The relation between the reaction and the oxygen-combining power of blood.*

Lastly there is the question of the relation between the reaction of blood and its oxygen-combining power as indicated by the value of log $K$ in Hill's equation to the oxygen dissociation curve. This subject was first of all investigated by Barcroft and Peters (7) who found that the curves showing the change in the values of log $K$ and of $p_H$ for Barcroft's blood with increasing $CO_2$-tension were superimposable, which indicated that the two quantities were proportional to one another throughout. Hasselbalch expressed this relationship by reading off the corresponding values of log $K$ and of $p_H$ at the same $CO_2$-tensions and plotting them against one another to obtain the straight line which he termed the Peters-Barcroft ("P.-B.") curve. It should be pointed out that, in obtaining this curve, Barcroft and Peters appear not to have carried out their log $K$ and their $p_H$ determinations on the same sample of blood, and, furthermore, Peters expressly states that he worked with an uncalibrated calomel electrode. Hasselbalch raised the question whether the P.-B. curve was of general application and expressed the relation between the blood-reaction and the value of $K$ for all individuals. He quotes cases from the literature in which this appears to be unlikely, but, using his own method of calculating the $p_H$'s from the $CO_2$-dissociation curve he obtained results with his own blood in accordance with the original P.-B. curve, and found also that during the changes in properties of woman's blood accompanying parturition the relation between acidity and oxygen-combining power showed no certain variation.

We have constructed the "P.-B." curves for the bloods we have examined: they are exhibited in Fig. 2 in which each diagram shows the curve for the particular man's blood before and after the oxygen treatment together with a broken line representing the *original* P.-B. curve. It will be seen at once that the curves we have obtained are all straight lines (within the limits of our experimental error) showing that in all of

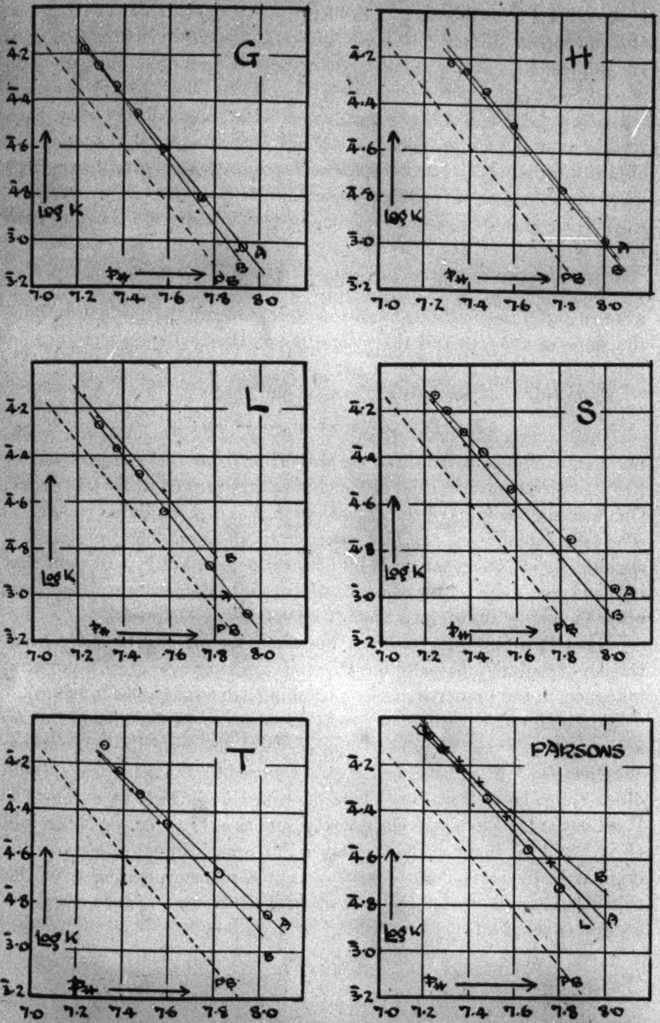


Fig. 2. *A* refers to the curve obtained after the oxygen treatment. *B* refers to the curve obtained before the oxygen treatment. *P.B.* represents the original P.-B. curve. *L* in the diagram for Parsons' blood refers to the curve obtained with the blood to which ·05 p.c. lactic acid had been added.

these cases the values of $p_H$ and of log $K$ are proportional to each other. But our curves do not coincide in position or exactly in direction with the original P.-B. curve, and, further, there is a marked, though by no means constant, difference between the curves obtained for the same individual before and after the oxygen treatment. There may be a certain amount of significance in the fact that where the two curves so obtained for any individual are markedly different they tend to approximate in the region of the physiological limits ($p_H$ 7·4 to 7·2).

We can, of course, represent such a system of straight lines as we have here by the general formula

$$\log K = \alpha p_H + \log \beta,$$

where $\alpha$ and $\beta$ are constants for any sample of blood, the first determining the slope of the line and the second its position with respect to some fixed line.

From this equation it is at once evident that

$$K = \beta (C_H)^{-\alpha} \text{ since } p_H = -\log C_H;$$

*i.e.* the equilibrium constant is proportional to some power of the hydrogen-ion concentration—a conclusion first arrived at by **Barcroft**. Our measurements have shown that both $\alpha$ and $\beta$ are liable to vary to a certain extent from one blood sample to another. The physiological significance of these changes is by no means clear, but it is evident that in blood, the dissociation constant of oxyhæmoglobin depends on some other factors in addition to the hydrogen-ion concentration.

**Hasselbalch**(8) suggests that one such factor may be the concentration of sodium-ions—and metallic-ions are known to exert a powerful influence on the properties of hæmoglobin (**Barcroft** and **Camis**(9)).

So far in our experiments on the P.-B. curve all the changes of reaction of the blood have been produced by changing its $CO_2$-tension. Whether the effects of changes of reaction produced in other ways would differ appreciably from those here described is difficult to decide: the P.-B. curve for **Parsons'** blood containing about ·05 p.c. of lactic acid ($L$ in Fig. 2) is found to be a straight line practically parallel with the original P.-B. curve, but as we have not the corresponding curve for this blood sample before the acid was added to it we cannot draw any general conclusion.

## SUMMARY.

1. The value of the reduced $p_H$ of the bloods of a number of individuals examined was found to be 7·37 ± ·01.

2. In one case the value of the reduced $p_H$ of the blood of a normal subject gradually changed from a value of 7·41 to 7·36 during the course of about nine months: the change was not accompanied by any disturbance of respiration.

3. The value of the $p_H$ of a blood sample at any $CO_2$-tension calculated from the $CO_2$-content at that tension is found to be lower than that observed experimentally: the divergence is probably due to the difference in the behaviour of the corpuscles and of the plasma with respect to $CO_2$-combining power.

4. The changes produced by alterations of the $CO_2$-tension in the values of $p_H$ and of $\log K$ in Hill's equation are always directly proportional to each other: but the exact ratio between them varies from one blood sample to another.

We are indebted to Mr Barcroft for much helpful criticism during the prosecution of this research, and to Mr G. S. Adair of King's College for assistance with our experiments.

The work was carried out during the tenure of a Travelling Medical Scholarship of the National University of Ireland by J. F. D. and of a research studentship at Sidney Sussex College by T. R. P. This paper forms a report to the Medical Research Committee to whom also our thanks are due for a further grant enabling us to carry on our investigations.

## REFERENCES.

(1) **Hill.** Biochem. Journ. 7. p. 471. 1913.
(2) **Hasselbalch.** Biochem. Ztschr. 78. p. 112. 1916.
(3) **Parsons.** This Journ. 51. p. 440. 1917.
(4) **Hasselbalch.** Biochem. Ztschr. 74. p. 56. 1916.
(5) **Campbell, Douglas and Hobson.** This Journ. 48. p. 301. 1914.
(6) **Buckmaster.** This Journ. 51. p. 164. 1917.
(7) **Barcroft and Peters.** Phil. Trans. B. 206. p. 69. 1914.
(8) **Hasselbalch.** Biochem. Ztschr. 82. p. 282. 1917.
(9) **Barcroft and Camis.** This Journ. 39. p. 118. 1909.

# FATS AND FATTY ACIDS AS FOOD. BY A COMMITTEE OF THE ROYAL SOCIETY.

CONTENTS.

PAGE

I. Introductory by W. D. Halliburton and D. Noël Paton . 328

II. Preparation from oils by J. C. Drummond, with Addendum by W. D. Halliburton . . . . . . 330

III. Feeding experiments on man by D. Burns and J. S. Sharpe . . . . . . . . . . . . 333

IV. Feeding experiments on animals by J. C. Drummond and W. D. Halliburton . . . . . . . . 339

V. The fat-soluble accessory factor by J. C. Drummond . 344

## I. INTRODUCTORY. BY W. D. HALLIBURTON AND D. NOËL PATON.

In the last months of 1917, the marked shortage in the supply of fats led to a reduction of the fat ration to about 3·2 ounces of butter, margarine and lard per week per person, while the limitation in the meat ration reduced the fat supplied in this way to some 3½ oz. or less. At most therefore, even allowing for fat in milk and other food stuffs, probably only about 7 or 8 oz. per week were available. The minimum adequate allowance generally considered necessary is about 70 grm. per day or 490 grm. (17 oz.) per week for the average member of the community requiring 2500 calories per day. The reason of the fat shortage was not far to seek, for fats, formerly used as food, were split in the preparation of glycerol for the manufacture of explosives; the fatty acids liberated simultaneously do not appear to have been used as human food.

The Food (War) Committee of the Royal Society was consulted, and the possibility of drawing upon fresh sources had to be considered. We were appointed a small sub-committee to investigate the problem, and our main enquiry was directed to the possibility of utilising as human food the fatty acids just referred to. It was estimated that some 100,000 tons a year of these fatty acids could be set free for the purpose, if they were found to be suitable. This would mean a substantial gain to the fat supply of the nation, which in pre-war time was estimated at 1,651,000 tons, an amount far above the actual requirement, for fat more than any other food constituent was extensively wasted. We also took into account the fact that even if fatty acids could not be used by man, it was possible that they might be used by animals, and thus fat suitable for human food would be set free.

In carrying out this work, we have to acknowledge valuable assistance from many helpers. The following accounts are taken from our Reports. They show that fatty acids prepared as above may be utilised for human food. Whether in the future it will be economically desirable to do this has yet to be decided. The reports on the digestibility of bread which are quoted several times in what follows, will soon be published.

In attempting to assess the value of fatty acids as food, four questions had to be considered:

1. The extent to which they could be made available, if found of value as human food.
2. Their digestibility in the human subject.
3. The effect of their continued use upon health.
4. How far they can be made palatable.

At first we had considerable difficulty in getting definite information as to the extent to which fatty acids were available. Mr Mansfield, the Director of the Oils and Fats Branch of the Ministry of Food, informed us that various trades were eager to obtain the stocks of fatty acids on hand; soaps, candles, paints and varnishes, all require fats. Mr Mansfield, however, put us into touch with Mr Tulloch of the Olympia Oil Mills, Selby, who has most obligingly given us all the assistance in his power, and to whom we desire to express our thanks, both for valuable information and for the material with which our experiments have been conducted. We would couple with his name that of Mr W. E. Ryall, one of the chemists in charge.

Before starting our investigations, we felt it necessary that a visit should be made to the Selby Mills, and we were fortunate to find that Dr Drummond could do this for us; his report (abbreviated) forms the next paper of this series.

The questions referred to under heads 2 and 3, digestibility and effect upon health, were carried out simultaneously in Glasgow and London, respectively; in the former place, the experiments on man were performed, and in the latter those on animals. These form the subjects of Papers III and IV.

The fourth question, namely that relating to palatability, was taken up by Mr Leitch of the West of Scotland Agricultural College, and his method is shortly described in Paper III.

The study of fat in diet opens up many wide questions, such as the magnitude of a minimum supply compatible with health, and the so-called iso-dynamic value of fat and carbohydrate; into these we do not intend to intrude. We may say, however, that the work has led us into several by-paths where research is still in progress. One of these for example dealt with cocoa-butter as a substitute for butter and will in due time be reported on by Drs Gardner and Fox; another with the "fat soluble accessory factor"; a preliminary note by Dr Drummond, which forms the concluding paper of this series, treats of some aspects of this important substance.

## II. PREPARATION FROM OILS. BY J. C. DRUMMOND, D.Sc.

At present very large quantities of hardened, *i.e.*, hydrogenated, oils and fats are being used for edible purposes. If these oils are split up to obtain glycerol the question arises whether the resultant fatty acids are also suitable for food.

The Selby Works treat a large number of oils, of which the chief are: whale oil, seal oil, palm-kernel oil, palm oil, coconut oil, sesame oil, soya oil, cottonseed oil, linseed oil and arachis oil. Practically all these oils are hardened to various degrees prior to saponification. Hardening is effected at 250° C. at atmospheric pressure by hydrogen gas operating in the presence of a nickel suboxide catalyst. Saponification is carried out by a contact method based upon a modified Twitchell process. Hardening can be arrested at any stage, it being thereby possible to produce at will a fat of any required melting point.

The characteristic, and frequently unpleasant, odour and taste of many oils, such as whale oils, are removed at an early stage in the hydrogenation. The process is also carried out in such a manner that the excess of hydrogen leaving the oil carries with it volatile impurities, such as the lower members of the fatty acid series, which may be present in the free state. It is, therefore, not infrequent to find that the hardened fat has a lower free acidity than the original oil.

The hardened fats are usually almost pure white solids, possessing melting points about 20–40° C. This can be controlled at will. They are odourless and practically tasteless. Free acid is present in very small amount, whilst traces of the nickel catalyst are present to an extent of less than one part in 10,000,000.

The fatty acids obtained after splitting prior to purification are usually of a pale sandy colour. They are free from smell and possess melting points varying between 15° and 45° C. This may also be controlled during the hardening of the parent oil, since the melting point of the fatty acids obtained from a hardened oil is usually higher than that of the oil itself.

The crude fatty-acid mixtures usually contain some 10 p.c. of unsaponified fat, due to the fact that it is unremunerative to press the saponification beyond about 90 p.c. Unsaponifiable matter of the cholesterol and phytosterol type is also present. The impurities present in the crude acids are mainly those introduced by the contact reagent used during saponification, *i.e.*, traces of "mineral oil" and sulphuric acid (actual process and nature of "mineral oil" are secret). Sulphuric acid is readily removed by barium carbonate treatment. This is done for the sake of cheapness, but in view of the possible toxicity of barium salts, it would be better to use calcium or magnesium carbonates for purifying fatty acids intended for edible purposes.

After discussion of the various points referred to above, with Mr Tulloch and Mr W. E. Ryall, the conclusion was reached that the following products, the supply of which is assured, were those most suitable for study:

1. Fatty acids from hardened whale or seal oil.
2. " " palm or palm-kernel oil.
3. " " "soy-cot" oil.

The latter oil is an oil much used for hydrogenation by the Selby Works, on account of the satisfactory character of the products it yields. Its actual composition was not given to me, but I understand that it is a mixture of palm-kernel, soy-bean, and cottonseed oils.

Mr Tulloch informed us later that palm-kernel oil is pretty well saturated already; hardening it is therefore not necessary unless it happens to form part of a mixture such as "soy-cot" oil.

## ADDENDUM. By W. D. HALLIBURTON.

The ethyl ester from palm-kernel oil has been prepared on a small scale in my laboratory by Dr O. Rosenheim. The unpleasant taste and odour of the original material disappeared and the product had a distinctly pleasant (alcoholic) flavour. This taste, and its low melting point (due probably to admixture with lower fatty acids), are much against its use in margarine manufacture, to say nothing of the expense involved in employing large quantities of alcohol, if the process was carried out on a large scale. Moreover such esters are not so readily absorbed as are ordinary fats, or their free fatty acids. According to Lyman's (1) investigations on two dogs, while glycerol palmitate gave a utilisation value of 94 to 95 p.c., the figure for ethyl palmitate was only 57 to 59 p.c.

Franck (2) states that in Germany esters have been prepared from fatty acids. The glycol ester was found to be the most suitable for food purposes; dogs and men were fed on it for four-day periods without ill-effects. In the dog, the utilisation figure was 90, and in man 77–80 p.c., which is higher than had previously been found for the ethyl esters.

Among the impurities of the hydrogenated fats mentioned in Dr Drummond's report is nickel; this, however, is only present in minute amount. Professor Cushny informed us that nickel is not highly toxic, and that the amount present is well within the limits of safety. Professor Cushny's opinion runs: "Minute traces of nickel in hydrogenated fats are not injurious to health. Nickel is among the most harmless of metals and I am unaware of any case of poisoning having occurred from it, although several investigators have taken considerable quantities of it in food often over prolonged periods. It is difficult, therefore, to name a definite figure as a limit, but it may be suggested that if the fat should be found to contain more than 10 parts per million, careful observations should be made. My own impression is that fat containing very large amounts of nickel would have an unpleasant metallic taste, and that this would prevent the use of any fat containing an injurious amount of nickel."

Among the impurities not mentioned is arsenic, and as the possibility of its presence was raised on the Food (War) Committee, I obtained samples of the reagents used and the products from Selby, and submitted them to Mr John Webster, F.I.C., of the Pathological Department, St Mary's Hospital, London, who has kindly furnished a report on the subject.

It is not necessary to give his results for the reagents, but as regards the products, one specimen of linseed oil after treatment contained 2·5 parts of arsenic ($As_2O_4$) per million, but the fatty acids obtained from it contained only 0·5, and the hardened whale oil fatty acids 1 part per million.

As a matter of fact, no signs of arsenic poisoning occurred, either in the men or animals who formed the subjects of experiments. Nevertheless, this is a danger to be borne in mind and guarded against, should it ever be necessary to employ fatty acids obtained by the use of mineral acids in our food supply. Professor **Cushny** wrote to me "I should say that one part per million of $As_4O_6$ in fats is quite harmless and probably much less than that taken in several kinds of food."

### REFERENCES.

(1) **Lyman.** J. Biol. Chem. **32.** p. 7. 1917.
(2) **H. Franck.** Münch. med. Wochensch. Oct. 1918, **65.** p. 1216.

## III. FEEDING EXPERIMENTS ON MAN. By D. BURNS and J. S. SHARPE.

*(From the Physiological Laboratory, University of Glasgow.)*

The following is a brief survey of the principal work on the digestibility of fatty acids performed previous to the present research.

**Munk**(1), in his classical researches published in 1880 and 1884, showed that neutral fats are split in the intestine into free fatty acids and glycerol, and that after absorption they are recombined to form neutral fats. He further showed that the free fatty acids are absorbed and combined with glycerol in the body to form neutral fats, and that the fatty acids exercise the same protein-sparing action as the equivalent amount of fats. One dog was kept for no less than 21 days on fatty acids(2), instead of fats, without disturbance of its metabolic balance. In 1890 he(3) demonstrated on a patient suffering from a lymphatic fistula that a fatty acid, erucic acid, is absorbed from the human intestine and combined with glycerol to form the neutral fat. **Munk's** results were confirmed by **Walther**(4) on dogs with a fistula of the thoracic duct and by **Frank**(5) in 1894.

**Levites**(6) investigated the absorption of fatty acids and their sodium salts on dogs with iliac fistulæ and showed that the absorption varies inversely with the melting point of the fat.

More recently **Lauder** and **Fagan**(7) have published the results of feeding pigs with the fatty acids of coconut oil. The check diet consisted of maize meal, sharps and green food, and 5 p.c. was replaced by fatty acids. As much as 9·5 oz. were given to pigs weighing from 32 to 68 lb.

The pigs gained weight to the same extent as the checks and showed no symptoms.

**Lyman** (8) found in dogs that while glycerol palmitate was absorbed to 95 p.c., palmitic acid was absorbed to 82 p.c. In rats he found that palmitic acid was laid on in the body as tripalmitate. In dogs the fatty acid proved an irritant and the food left the stomach more slowly.

We have been informed that Messrs Bibby & Co., of Liverpool, have also carried out experiments upon the subject upon cows and pigs, which show that the fatty acids are well utilised, and that they are in no way prejudicial to health. In the case of the cows as much as half a pound (227 grm.) of the fatty acids of whale oil was given per day while the pigs received 3·2 oz. (92 grm.).

Upon the digestibility of hardened fats experiments have been recorded by **Thoms** and **Müller** (9), by **Süssmann** (10), by **Smith, Miller** and **Hawk** (11), and by **Pekelharing** and **Schut** (12). These experiments, especially those of the American observers, show that the hardened fats are well digested and absorbed by the human subject.

In the face of such results, it might appear almost unnecessary to test directly the digestibility of fatty acids in man. But it was felt that only after such a direct test had been applied would it be possible to recommend their use as a human food.

Animal experiments have hitherto been mainly performed upon dogs, but it was considered necessary to supplement these with experiments on rats (see next paper). Three or four weeks for a dog is not a very large portion of its life, and if the effect of the continued use of fatty acids is to be adequately determined, some animal such as the rat must be selected in which a few weeks form a larger proportion of its total life-span.

The first sample received was of the fatty acids of palm-kernel oil. It had a most disagreeable taste and a strong acid reaction. It was found to have been imperfectly neutralised with barium carbonate, and it contained a fair proportion of sulphuric acid. It was impossible to use it for experiments on the human subject, but it was used in experiments upon rats (see Paper IV). After correspondence, Mr Tulloch kindly arranged that further samples should be more carefully neutralised, and that calcium carbonate should be used for the purpose.

The next sample of fatty acids received was prepared from hardened whale oil. Its flavour was still unpleasant, but after consultation with Mr Leitch, of the West of Scotland Agricultural College, we succeeded (by the process described later) in making it sufficiently palatable to be eaten as "butter" on bread or mashed with potatoes[1]. It is upon this product that the experiments upon digestibility have been made.

The most palatable of the fatty acids sent is that prepared from hardened "soy-cot" oil, which, however, was received after experiments on whale oil were already in progress.

Our experiments were carried out upon three healthy men, two of whom had been subjects in previous investigations on the digestibility of bread. They were arranged to give a comparison between the digestibility of the fatty acids and the hydrogenated fat from which they had been split in order to answer the question of whether these fatty acids after splitting off the glycerol are actually available as human food.

Each subject was put upon a fixed diet, the composition and energy value of which was directly determined. A diet containing a small amount of ordinary fats was purposely selected in order that the fatty acids or hardened fats should form a very considerable proportion, so that any failure in absorption should not be masked by the perfect absorption of the other fats.

For this reason and to make the test of their digestibility more crucial the amounts given were large—36 grm. of the fatty acids, equivalent to about 40 grm. of fat, and 46·5 grm. of the hardened fat, constituting about 70 p.c. of the total fat intake. It is not likely that such amounts would ever be consumed, but the addition of 5 or 6 oz. per week to the present supply of fat would make a most substantial contribution to the energy intake of the diet.

The hydrogenated whale oil had a melting point of 38·5° C., was white in colour like tallow, hard like mutton fat in consistency, had a slight odour like tallow, and was tasteless till melted, when it tasted like lard. The fatty acids prepared from it melted at 40° C., were yellow like brown sugar in colour, and were friable, more like dripping in consistency. The smell was more pronounced than that of the original fat, and the taste resembled that of roast dripping. The colour darkened in melting, and the odour became more pronounced. The hydrogenated fat contained about 8·5 p.c. of glycerol.

To increase the palatability of both the fatty acids and the fat they were prepared in the following manner:—Two parts, by weight, of the melted fat or fatty acid were switched up with one part of soured milk, the whole being kept at a temperature above the melting point of the fat. The emulsion so formed was sprayed into a large volume of whey at the same temperature. This mixture was thoroughly agitated on a mechanical shaker and allowed to stand overnight in ice. The "butter" was removed from the top of the whey, excess of water removed by pressure and by beating with butter paddles. It was then salted to taste.

---

[1] As regards palatability, much remains to be done, and up to the present samples obtained through other sources prepared by the lipase method appear more promising than those prepared by mineral reagents. D. N. P. and W. D. H.

When prepared from hardened whale oil it was quite palatable and was taken as "butter" spread on bread or mashed with hot potatoes.

On the other hand the result of treating the fatty acids from whale oil by the above process was not quite so satisfactory. The "butter" had a slight smell and flavour of dripping, and at first the subjects preferred to mask the flavour by spreading fruit-jelly over the "buttered" bread, and one subject took it with mashed potatoes. Before the first significant day of the experiment they had become quite reconciled to the smell and flavour and partook of the "butter" without camouflage.

TABLE I. Composition of Diet.

| Foodstuff | Amount per day in grams | Percentage composition | | | E.V. per 100 grm. (in Cals.) |
|---|---|---|---|---|---|
| | | Moisture | N. | Fat | |
| Bread ... ... ... ... | 500 | 46·8 | 2·32 | 0·65 | 414·5 |
| Café au Lait ... ... ... | 90 | 25·03 | 1·41* | 4·8 | 466 |
| Chocolate* ... ... ... | 30 | ... | 1·33 | 36·15 | 587 |
| Consommé ... ... ... | 6 | 8·6 | 6·2* | ... | 187 |
| Jelly ... ... ... ... | 50 | 30 | 0·14* | ... | 468 |
| Pine Apple ... ... ... | Fruit 60<br>Juice 20 | 71·5 | 0·07* | 0·7* | 348 |
| Potatoes ... ... ... ... | 200 | 75·65 | 1·15 | 0·1 | 380·2 |
| Rice and milk (sweetened) ... | 200 | 76·2 | 1·42 | 0·4 | 387 |
| Meat powder† ... ... ... | 30 | 6·7 | 12·3 | 7·457 | 478·3 |
| Fatty acid (butter) ... ... | 60 | 50 | 0·64 | 50* | 560·5* |
| Fat (butter) ... ... ... | 50 | 19·2 | ... | 80·8* | 767* |

* Analysed undried, as used.

† Cole's figures—Food Committee (Roy. Soc.), No. 42.

TABLE II. Total Intakes per Day.

(a) Fatty acid period.

| Subject | N. in grm. | Fat in food in grm. | Added fatty acid in grm. | Total fat in grm. | E.V. in Calories |
|---|---|---|---|---|---|
| J. R. T. ... ... ... ... | 13·73 | 16·8 | 36 | 52·8 | 2689·6 |
| D. L. ... ... ... ... | 14·96 | 17·15 | 36 | 53·15 | 2908·6 |
| J. W. ... ... ... ... | 14·91 | 17·13 | 36 | 53·13 | 2906·4 |
| (b) Hardened fat period. | | | | | |
| J. R. T. ... ... ... ... | 13·25 | 16·8 | 45·3 | 62·1 | 2787·9 |
| D. L. ... ... ... ... | 13·25 | 16·8 | 45·3 | 62·1 | 2787·9 |
| J. W. ... ... ... ... | 13·25 | 16·8 | 45·3 | 62·1 | 2787·9 |

### *Notes on the Articles of Diet.*

(*a*) **Bread.**—The baking of the bread was carried out by the experimental staff of the Scottish Co-operative Wholesale Society, Ltd., at the Regent Mills, Glasgow, under the personal supervision of Mr Sproul, to whom our thanks are due. The bread was made entirely from flour of 88 p.c. extraction. No other flour was added. As the outsides of the loaves showed traces of oil they were removed before the bread was weighed.

(*b*) **Meat powder.**—This was drawn from a supply kindly sent to us by Prof. F. G. Hopkins, and reported on by Mr Cole in paper 42, Food (War) Committee, Royal Society.

(*c*) **Café, chocolate, jelly and consommé,** were similar to those used in the studies on the "Digestibilities of Breads," Food (War) Committee, Royal Society. Fresh analyses were made.

*Method of Experiment.* The diet was consumed in two periods of six days each with an interval of a month between these periods during which the subjects returned to an ordinary dietary. No special demarcation of the fæces was attempted but the urine of the first two and the fæces of the first three days of each period were excluded from the final calculation.

The daily ration was weighed out at the same time each day. The entire crust of the pan loaf was removed and 500 grm. of the crumb rapidly weighed for each subject. A centre slice from each day's bread was at the same time taken for analysis.

The collection of the excreta was carried out exactly as in the bread studies already referred to.

The fæces were dried at 80° C., powdered and extracted with hot methylated ether after treatment with HCl to break up soaps. To ascertain whether any loss of fat occurred in this method, the following observation was made:—A moist stool was well mixed and two portions of 10 grm. taken. One portion was immediately treated by the Gephart-Csonka method, while the other portion was dried and extracted by the method employed throughout this study. The fat content was

(*a*) Gephart-Csonka = 6·6 p.c. (*b*) Extraction method = 7·7 p.c.

It thus seems manifest that there is no loss of fat through drying the fæces before extraction.

Nitrogen was estimated by Kjeldahl's method, and E.V. by bomb calorimeter.

TABLE III. Utilisation per Day.

(*a*) Fatty acid period.

| Subject | Total fat intake | | Fat in fæces | Fat absorbed | Per cent. of fat absorbed |
|---|---|---|---|---|---|
| | grm. | | | | |
| J. R. T. | 52·8 | 36 grm. fatty acids | 4·7 | 48·1 | 91·0 |
| D. L. | 53·15 | | 6·0 | 47·15 | 88·7 |
| J. W. | 53·13 | | 4·19 | 48·94 | 92·1 |

TABLE III (*contd.*).

(*b*) Hardened fat period.

| Subject | Total fat intake | | Fat in fæces | Fat absorbed | Per cent. of fat absorbed |
|---|---|---|---|---|---|
| | grm. | | | | |
| J. R. T. | 62·1 | 45·3 grm. hardened fat | 5·49 | 56·6 | 91·2 |
| D. L. | 62·1 | | 6·26 | 55·9 | 90·0 |
| J. W. | 62·1 | | 6·21 | 55·9 | 90·0 |

Average percentage of total fat ingested given in form of—

1. Whale oil fatty acid = 68 p.c. (= 74 p.c. fat).
2. Hardened whale oil = 72 p.c. (= 66 p.c. fatty acid).

These results show a somewhat lower digestibility than was found by Smith, Miller and Hawk (*loc. cit.*) for hydrogenated cottonseed oil with a melting point of 36° C.

TABLE IV. Energy Utilisation of Diet per Day in Calories.

(*a*) Fatty acid period.

| Subject | Energy intake | Energy in fæces | Energy used | Per cent. of energy used |
|---|---|---|---|---|
| J. R. T. | 2689·6 | 110·1 | 2579·5 | 95·9 |
| D. L. | 2908·6 | 128·4 | 2780·2 | 95·6 |
| J. W. | 2906·4 | 104·65 | 2801·75 | 96·4 |

(*b*) Hardened fat period.

| Subject | Energy intake | Energy in fæces | Energy used | Per cent. of energy used |
|---|---|---|---|---|
| J. R. T. | 2787·9 | 106·26 | 2681·64 | 96·2 |
| D. L. | 2787·9 | 123·3 | 2664·6 | 95·6 |
| J. W. | 2787·9 | 103·3 | 2684·6 | 96·3 |

TABLE V. Utilisation of Nitrogen of Diet per Day in Grms.

(*a*) Fatty acid period.

| Subject | Alteration in weight for the whole period of six days | Nitrogen intake | Urine | Fæces | Balance | Absorbed | Per cent. used |
|---|---|---|---|---|---|---|---|
| | kilo. | | | | | | |
| J. R. T. | −1·0 | 13·73 | 13·03 | 0·86 | −0·14 | 12·87 | 93·7 |
| D. L. | ±0 | 14·96 | 13·30 | 1·24 | +0·42 | 13·72 | 91·2 |
| J. W. | −0·8 | 14·91 | 13·96 | 1·10 | −0·15 | 13·81 | 92·6 |

(*b*) Hardened fat period.

| Subject | Alteration in weight for the whole period of six days | Nitrogen intake | Urine | Fæces | Balance | Absorbed | Per cent. used |
|---|---|---|---|---|---|---|---|
| J. R. T. | −1·2 | 13·25 | 9·06 | 0·85 | +3·34 | 12·4 | 93·6 |
| D. L. | ±0 | 13·25 | 11·54 | 1·62 | +0·09 | 11·63 | 87·7 |
| J. W. | −0·3 | 13·25 | 10·96 | 1·25 | +1·04 | 12·0 | 90·6 |

The addition of neither hardened fat nor of fatty acids to the standard diet had any adverse effect on the health of the subjects. The loss of weight experienced by two of them, J. R. T. and J. W., on both diets may be explained satisfactorily by the fact that the nitrogen and energy value of their diet was less than they had been accustomed to and considerably less than in the previous bread experiments where J. R. T. had 3376 calories and J. W. 3964 calories. Their weight returned to normal within three or four days of stopping the experiment. None of the subjects complained of alimentary trouble. Defæcation was normal. There was no flatulence or regurgitation from the stomach.

## CONCLUSIONS.

1. The fatty acids of hardened whale oil in amounts of 36 grm. per diem, or more than one-third of the usual intake of fat, were as thoroughly digested and absorbed as was the hardened oil from which they were prepared.
2. Their administration for a period of six days caused no disturbance in the absorption of the other constituents of the food as is indicated by the nitrogen and energy balances, nor any other disturbances in health.
3. They may, therefore, be used as a source of fat in the diet of man.

## REFERENCES.

(1) **Munk.** Virchow's Archiv, **80**. p. 10, 1880; **95**. p. 407. 1884.
(2) **Munk.** Loc. cit. **80**. p. 20.
(3) **Munk.** Arch. (f. Anat. und) Physiol. pp. 376, 581. 1890.
(4) **Walther.** Ibid. p. 329. 1890.
(5) **Frank.** Ibid. p. 297. 1894.
(6) **Levites.** Zeitsch. physiol. Chem. **53**. p. 349. 1907.
(7) **Lauder and Fagan.** J. Soc. Chem. Industry, **36**. No. 20. 1917.
(8) **Lyman.** J. Biol. Chem. **32**. pp. 7–13. 1917.
(9) **Thoms and Müller.** Arch. f. Hygien. **84**. p. 54. 1915.
(10) **Süssmann.** Ibid. p. 121. 1915.
(11) **Smith, Miller and Hawk.** J. Biol. Chem. **23**. p. 505. 1915.
(12) **Pekelharing and Schut.** Arch. néerl. de physiol. **1**. p. 635. 1917.

## IV. FEEDING EXPERIMENTS ON ANIMALS.

BY J. C. DRUMMOND AND W. D. HALLIBURTON.

*(From the Physiological Laboratory, King's College, London, and Research Institute, Cancer Hospital, London.)*

For the reasons stated in Paper III, rats were used in the following experiments. The fatty acids from palm-kernel oil, hardened whale oil and "soy-cot" oil have been investigated. The experiment with the first was a very drastic one, for the specimen first used contained more impurities than those sent subsequently, and its taste and odour rendered it unsuitable for human food. It also contained barium sulphate and sulphuric acid and lower fatty acids; nevertheless the animals took it eagerly and throve upon it when their diet contained an admixture of 5 p.c. A 10 p.c. admixture was not quite so favourable, but even then nothing tangibly harmful could be pointed to.

*Diet I.* Five p.c. palm-kernel oil fatty acid ration.

| | |
|---|---|
| Purified caseinogen ... ... ... ... | 18 parts |
| „ starch ... ... ... ... | 52 „ |
| Salt mixture ... ... ... ... ... | 5 „ |
| Butter fat ... ... ... ... ... | 15 „ |
| Fatty acids from palm-kernel oil ... ... | 5 „ |
| Yeast extract ... ... ... ... ... | 5 „ |

The composition of the mixture of inorganic salts used may be found in a previous paper by ourselves (1). The six rats upon this ration showed a satisfactory consumption of food, and, with the one exception noted in the table, remained in good health throughout the experiment. (Table I.)

TABLE I.

| | Weight of rats in grms. | | | | | | Food consumed in week g. |
|---|---|---|---|---|---|---|---|
| Date | 1 | 2 | 3 | 4 | 5 | 6 | |
| 8. iii. 18 | 213 | 260 | 223 | 160 | 137 | 170 | — |
| 12. iii. 18 | 220 | 260 | 220 | 161 | 140 | 174 | 670 |
| 19. iii. 18 | 224 | 285 | 239 | 165 | 155 | 187 | 710 |
| 26. iii. 18 | 231 | 300 | 244 | 137 | 177 | 190 | 615 |
| 1. iv. 18 | 236 | 315 | 270 | 159 | 197 | 200 | 800 |
| 8. iv. 18 | 240 | 310 | 275 | 155 | 200 | 202 | 590 |
| 15. iv. 18 | 240 | 310 | 280 | 165 | 200 | 206 | 655 |
| 22. iv. 18 | 248 | 320 | 285 | 174 | 210 | 214 | 705 |
| 29. iv. 18 | 250 | 310 | 285 | 170 | 215 | 220 | — |
| 8. v. 18 | 252 | 300 | 300 | 180 | 219 | 224 | — |
| Increase in 2 months | 39 | 40 | 77 | 20 | 82 | 54 | |

From 8. iii. 18 to 25. iii. 18 the sample of fatty acids employed was the highly unpalatable preparation referred to on p. 334. From 25. iii. 18 to the end of the experiment a more refined preparation was used.

Rat 4 poorly between 19. iii. 18 and 8. iv. 18. Cause undetermined. Recovery complete.

The average weekly consumption of the diet was 680 grm. Allowing for water added to render the diets pasty, this represents approximately 570 grm. of the dry ration. A rough calculation may be made, which gives the daily intake of fatty acids as approximately 0·3–0·4 grm. per 100 grm. live weight.

*Diet II.* Ten p.c. palm-kernel oil fatty acid ration.

| | |
|---|---|
| Purified caseinogen ... ... ... ... | 18 parts |
| " starch ... ... ... ... | 52 " |
| Salt mixture ... ... ... ... ... | 5 " |
| Butter fat ... ... ... ... ... | 10 " |
| Fatty acids ... ... ... ... ... | 10 " |
| Yeast extract ... ... ... ... ... | 5 " |

Four rats have been fed upon this ration. Two of these died during the course of the experiment, one showing symptoms of pneumonia, the other from an undetermined cause. The other two animals showed fairly good growth, and appeared to be in a satisfactory state of health at the conclusion of the experiment. Food consumption showed a drop, owing to the declining health of the two animals which succumbed. An approximate estimate of the consumption of fatty acids in this group, based on the food consumption during the early period when all four animals were in good health, is 0·65 grm. per 100 grm. live weight per day. The results are given in Table II.

TABLE II.

| Date | Weight of rats in grm. 1 | 2 | 3 | 4 | Weekly food consumption in grm. |
|---|---|---|---|---|---|
| 19. iii. 18 | 176 | 177 | 206 | 206 | — |
| 26. iii. 18 | 166 | 170 | 213 | 209 | 550 |
| 1. iv. 18 | 168 | 162 | 216 | 225 | 425 |
| 8. iv. 18 | 176 | 162 | 208 | 235 | 385 |
| 15. iv. 18 | 181 | 142 | 220 | 232 | 344 |
| 22. iv. 18 | 167 | 135 | 222 | 240 | 345 |
| 29. iv. 18 | 140 | — | 217 | 239 | — |
| 4. v. 18 | 136 | — | 219 | 242 | 225 |
| 8. v. 18 | — | — | 220 | 245 | 260 |
| Increase | — | — | 14 | 39 | |

Rat 2 died 24. iv. 18, pneumonia.
Rat 1 died 5. v. 18, cause undetermined.

It must be remembered that these four animals received a smaller percentage of butter fat in their ration than those receiving Diet I. Their somewhat sub-normal rate of growth and lowered resistance to disease may conceivably be due to the smaller amount of the fat-soluble accessory which they received.

*Diet III.* 5 and 10 p.c. hardened whale oil fatty acids diets.

Composition of diet similar to Diets I and II, with whale oil fatty acids instead of palm-kernel oil and fatty acids.

TABLE III.

| | Weight of rats in grm. | | | | | | | Weekly food consumption |
|---|---|---|---|---|---|---|---|---|
| Date | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| 26. iii. 18 | 192 | 186 | 226 | — | — | — | — | — |
| 1. iv. 18 | 184 | 180 | 205 | — | — | — | — | 280 |
| 8. iv. 18 | 182 | 179 | 202 | — | — | — | — | 285 |
| 15. iv. 18 | 185 | 188 | 216 | — | — | — | — | 290 |
| 22. iv. 18 | 194 | 188 | 221 | — | — | — | — | 290 |
| 29. iv. 18 | 195 | 186 | 232 | 134 | 118 | 67 | 76 | — |
| 6. v. 18 | 198 | 187 | 220 | 132 | 114 | 79 | 81 | — |
| 13. v. 18 | 198 | — | 225 | 137 | 123 | 102 | 92 | — |
| 20. v. 18 | 214 | — | 224 | 153 | 133* | 104 | 112 | — |
| 27. v. 18 | 206 | — | 210 | 143 | 99 | 118 | 122 | — |
| 3. vi. 18 | 215 | — | 190 | 146 | 113 | 140 | 145 | — |
| 10. vi. 18 | 218 | — | 219 | 157 | 120 | 158 | 168 | — |

* Diet from 26. iii. 18 to 29. iv. 18 contained butter fat 15 p.c., fatty acids 5 p.c.
,, 29. iv. 18 to 20. v. 18 ,, ,, 10 ,, ,, 10 ,,

From 26. iii. 18 to 29. iv. 18 animals received 5 p.c. fatty acid diet. From 29. iv. 18 onwards they were given 10 p.c.

Rat 2 died, pneumonia, 19. v. 18.

Rat 5 pregnant, bore litter on May 25, 1918, but devoured her young.

The results of this experiment are satisfactory.

Maintenance of the adult animals and growth of the young ones were both good. Female rats when under normal conditions do not devour their litters, but frequently do so when their dietaries exhibit deficiencies. It is possible that rat 5 was receiving a slight deficiency of the fat-soluble accessory factor.

*Diet IV*. 5 and 10 p.c. hardened "soy-cot" fatty acid diets.

Composition of diets as for Diets I and II.

TABLE IV.

| | Weight of rats in grm. | | | | | | Weekly food consumption |
|---|---|---|---|---|---|---|---|
| Date | 1 | 2 | 3 | 4 | 5 | 6 | |
| 20. iii. 18 | 190 | 205 | 144 | — | — | — | — |
| 26. iii. 18 | 188 | 183 | 138 | — | — | — | 265 |
| 1. iv. 18 | 196 | 187 | 143 | — | — | — | 290 |
| 8. iv. 18 | 192 | 192 | 143 | — | — | — | 270 |
| 15. iv. 18 | 215 | 220 | 151 | — | — | — | 320 |
| 22. iv. 18 | 215 | 220 | 150 | — | — | — | — |
| 29. iv. 18 | 230 | 229 | 159 | 76 | 66 | 61 | — |
| 6. v. 18 | 222 | 220 | 158 | 80 | 61 | 67 | — |
| 13. v. 18 | 230 | 225 | 160 | 95 | — | 72 | — |
| 20. v. 18 | 239 | 233 | 169 | 103 | — | 83 | — |

From 26. iii. 18 to 29. iv. 18 given 5 p.c. ration.

From 29. iv. 18 to 20. v. 18 given 10 p.c. ration.

Rat 5 died 7. v. 18, abscess under jaw.

Maintenance of adult rats and growth of young rats both appear to be satisfactory upon these rations.

*Diet V.* Growth of young rats upon diets containing 10 p.c. hardened "soy-cot" oil or hardened whale oil. (For comparison with growth of young rats on the 10 p.c. fatty acid rations given in Tables III and IV.)

Composition of diets similar to Diet II, 10 p.c. hardened "soy-cot" oil or hardened whale oil instead of 10 p.c. fatty acids.

TABLE V.

| | Weight of rats in grm. | | | | | |
|---|---|---|---|---|---|---|
| Date | 1 | 2 | 3 | 4 | 5 | 6 |
| 29. iv. 18 | 74 | 70 | 65 | 63 | 74 | 73 |
| 6. v. 18 | 84 | 85 | 77 | 73 | 80 | 82 |
| 13. v. 18 | 93 | 92 | 81 | 85 | 104 | 84 |
| 20. v. 18 | 103 | 103 | 85 | 100 | 116 | 80 |
| 27. v. 18 | 110 | 112 | 90 | 106 | 120 | 99 |
| 3. vi. 18 | 114 | 110 | 99 | 110 | 125 | 107 |
| 10. vi. 18 | 121 | 125 | 109 | 129 | 132 | 112 |

From 29. iv. 18 to 27. v. 18 animals given 10 p.c. hardened "soy-cot" oil.
From 27. v. 18 onwards animals receiving 10 p.c. hardened whale oil.

The rate of growth of these young rats receiving a ration containing 20 p.c. of fat, made up of equal parts of butter fat and hardened fat, is in no way superior to that exhibited by the animals which received a dietary containing 10 p.c. of butter fat and 10 p.c. of fatty acids derived from the same hardened fats. (Tables III and IV.)

Diets I–IV all possessed the somewhat unpleasant odour of the fatty acids, even when only 5 p.c. was present. The butter-fatty acid mixtures were unpalatable and did not possess an attractive appearance, the dark colour of the fatty acids masking the pleasant yellow colour of the butter.

No toxic action whatever was observed to follow the administration of the free fatty acids, even when the crude sample described on pages 334 and 340 (Table I) was employed.

## CONCLUSIONS.

1. There is no toxic action excited by an admixture of 5–10 p.c. fatty acids of the three fats used.
2. The animals on such a diet take their food readily, grow healthily, and in some cases have produced young while on the diet.

Both human and animal experiments coincide in showing that fatty acids may be used as a source of food.

## REFERENCE.

(1) **Halliburton and Drummond.** This Journ. 51. p. 235. 1917.

## V. THE FAT-SOLUBLE ACCESSORY FACTOR.

By J. C. DRUMMOND.

One result of war conditions has been the disappearance, to a large extent, of the old prejudice against margarine. This, however, is a circumstance that is not free from danger to the community, especially as the present day margarines, though in some cases of satisfactory flavour, are mainly made of vegetable fats. It should in the future be compulsory that all margarines should contain a sufficient amount, either of real butter or egg-yolk, or of oleo-oil (beef-fat) in order that they may be more adequate sources of the fat-soluble accessory factor. Otherwise serious damage to the health of the population, particularly of young children, will be the inevitable result.

The necessity of this factor to the adult is not so fully proved, but the following extract from a report on the subject prepared by Mr Hardy and Professor Halliburton to the Fats and Oils Committee of the Food Investigation Board appears to present a satisfactory view of the matter:

"Fats act as carriers of a certain substance of unknown chemical nature which is absolutely essential to the growth of the body. If it is withheld from young animals, growth ceases and the animals die. The influence of this 'growth factor,' characteristically associated with most animal fats, upon adult animals is still obscure. It is certain that adult animals can exist in health when this factor is almost completely removed from the diet, and therefore it may be said that it might be almost completely withheld from adult men without harm. But our present knowledge does not exclude this possibility, namely that 'growth factors' are necessary to the processes of repair following upon periods of excessive strain. Indeed the available facts point in this direction. For instance in the case of deficiency diseases such as scurvy, the quantity of vitamine which will keep a person who is leading a sedentary life in health is insufficient when active exertion is undertaken."

The fats and fatty acids dealt with in the present investigation were in the main of vegetable origin, and therefore the fat-soluble accessory substance has been absent. In the experiments it has been necessary to add a certain amount of animal fat to supply the deficiency.

One fat in the list, namely whale oil, however, contains it, and the opportunity has been taken to ascertain whether the processes to which it is subjected, namely hydrogenation and splitting, destroyed this important constituent.

Whale oil appears in the market in several grades. The oil used in a previous investigation by myself (1) was a low grade oil, but it proved to be rich in the fat-soluble factor. As it had been subjected to somewhat drastic treatment (involving high temperature) in preparation, it was concluded that the accessory substance was stable to heat. This appeared to confirm the experiments of Osborne and Mendel (2) on the treatment of butter fat with steam. Indeed it seems to have been generally accepted that this accessory substance is more resistant to heat than the anti-neuritic and anti-scorbutic vitamines. This may be so, but subsequent experiments at present in progress show that the fat-soluble accessory is more thermolabile than was previously supposed to be the case.

Mr D. H. Simpson, the Manager of Ardol, Ltd, Selby, kindly placed material at my disposal. It was a high-grade whale oil, pale yellow in colour and its odour not more unpleasant than that of cod-liver oil. On cooling, it deposited a considerable quantity of "stearin." The sample examined had been subjected to what is termed "pre-treatment," which is a process of refining the oil undergoes prior to hardening; the process briefly consists in removing free fatty acids with soda and subsequent clarification with Fuller's earth. Experiments on rats showed it to be a rich source of "Fat-soluble A."

A similar sample hardened by the usual process, involving exposure to hydrogen gas at 250° C., for 4–6 hours, was next examined. This product, a white fat resembling mutton tallow in taste and odour, was entirely deficient in "Fat-soluble A."

In order to test whether the destruction is the result of reduction of some unsaturated substance, or merely of the high temperature, the oil was next simply heated to 250° C. for four hours. It became slightly darker, in tint, and proved to be also destitute of "Fat-soluble A."

A recent paper by Steenbock and others (3) then made it necessary to examine the effects of lower temperatures, so samples of whale oil were heated to 100° and 150° C., for four hours respectively. In both cases the destruction of "Fat-soluble A" was almost complete. Preliminary experiments with butter indicate that temperatures of 75°–100° C. are sufficiently high if continued for several hours to cause destruction of "Fat-soluble A." This work, and also that on the effect of heat on the "Fat-soluble A" in wheat embryo which are in progress will be fully reported upon in due course, but in the meanwhile it must be recognised that high temperatures are deleterious, and that margarines made from hydrogenated animal fats, although they are easily digestible, have a calorie value of the usual magnitude and may be palatable, are nevertheless of inferior nutritional value, and resemble vegetable fats in being destitute of the fat-soluble accessory factor.

## REFERENCES.

(1) **Drummond.** This Journ. **52.** p. 95. 1918.
(2) **Osborne and Mendel.** J. Biol. Chem. **20.** p. 381. 1915.
(3) **Steenbock and others.** Ibid. **35.** p. 517. 1918.

# A CONTRIBUTION TO THE PHYSIOLOGY OF THE AQUEOUS HUMOUR. BY W. A. OSBORNE.

*(From the Physiological Laboratory, University of Melbourne.)*

THE aqueous humour presents the singular phenomenon of a body fluid surrounded by living tissue and yet containing so little protein that it may be regarded as practically protein-free. As is well known in physiological theory a cellular tissue if brought into contact with a saline solution containing no colloid, which could balance the protein of the cells, will imbibe fluid from the saline by virtue of the osmotic pressure of the protein. This will take place even if the saline has a higher osmotic pressure than the cell contents, though there may then be an initial reverse action. Connective tissue apart from its enclosed cells will adsorb more fluid from a saline than from the same saline *plus* protein. According to theory then the cells of the iris, at least the superficial cells, must be in a state of œdema unless they are separated from the aqueous humour by a water-proof membrane.

Another problem is that presented by the alleged higher osmotic pressure of the aqueous humour. No other fluid in the body except the urine retains for any length of time a hyperosmotic concentration. In the case of the urinary passages there is present a pluricellular mucous membrane of a special type. A hyperosmotic fluid could not be produced in the ciliary body or elsewhere by filtration only; its formation would imply the performance of work and would constitute a true secretion. But of course an iso-osmotic solution can be made hyperosmotic by the loss of water through evaporation or expression from a semi-permeable membrane.

A general study of this theory led me to test some of the text-book statements concerning the aqueous humour. The fluid was obtained from ox eyes brought rapidly from the slaughter house to the laboratory. The cornea of each eye was wiped dry and opened with a sharp scalpel, the eyeball being held so that the cornea looked downwards. Generally the aqueous humour of 18 ox eyes was pooled, though in the quantitative sugar estimations the fluid in some cases was aspirated by a hypodermic needle from the anterior chamber.

*Protein content.* The protein in fresh ox serum was estimated by the Scherer coagulation method after proper dilution. Another sample of the same serum was diluted with Ringer's fluid until it possessed a protein concentration approximately equal to that of the aqueous. For nephelometric estimations I found that adding an equal volume of 1 p.c. trichloracetic acid gave a suitable precipitate. Using this method with the Kober nephelometer-colorimeter[1] I found in two estimations that the protein content was 0·08 p.c.

*Total solids and ash.* 13·1011 grams of aqueous humour were placed in a tared platinum vessel and exposed for two hours to a temperature of 105° C. I did not, however, test whether constant weight had been attained. The residue weighed 0·1567 gm. = 1·26 p.c. total solids by weight. This residue was ashed over a low flame with the lid on the platinum vessel. The ash residue weighed 0·1083 gm. = 0·82 p.c. mineral matter by weight. This, it will be seen, is what one would expect if the humour were a simple filtrate from the blood.

*Sugar content.* Two $\text{cm.}^3$ of aqueous humour were aspirated direct from the anterior chamber or were taken from the pooled fluid. The reducing sugar was estimated by the Benedict method in which a standard glucose solution and not picramic acid was employed. Three estimations gave me 0·07 p.c., 0·09 p.c., and 0·12 p.c. sugar estimated as glucose. I gave this quantitative estimation to some advanced students as an exercise and their results were 0·06 p.c., 0·09 p.c., 0·14 p.c. and 0·14 p.c. Again, it will be observed, the sugar content is of the order expected in a blood filtrate.

*Refractive index.* With a Zeiss-Abbe refractometer ox serum at 7·1° C. gave the reading 1·3451. Aqueous humour at the same temperature gave 1·336. I took a quantity of ox serum in a test tube, immersed this for ten minutes in a water bath at 100° C. and determined the refractive index of the protein-free fluid that eventually separated from the coagulum. The reading at 7·1° C. was 1·336, *i.e.* exactly the same as the aqueous.

*Freezing point.* In determining the osmotic pressure by this method I employed the same Beckmann thermometer that I have used for the last thirteen years. I have always adjusted the mercury so that the meniscus at the freezing point should be close to the centre of the scale. The freezing mixture was separated from the fluid to be investigated by a layer of air as well as two glass walls. Inoculation with an ice crystal took place when the supercooling was about 0·75° C. Stirring was carried out by a clock-work mechanism. A too low temperature in the freezing mixture was avoided. With this technique I have taken from time to time the depression of freezing point of a number of ox blood specimens, some twenty-five to thirty in all, and invariably the first two figures of the decimal have been the same, namely 0·59° C. The experiment was carried out with the pooled aqueous humour from 18 ox eyes and the depression obtained was 0·597° C.

---

[1] It is a pity that this otherwise fine instrument should possess an error in construction. As pointed out to me by my colleague Professor Laby the containing vessels into which the plungers dip should be square in section and not circular as this latter shape gives lack of uniformity in each field.

### *Discussion.*

The results of these experiments confirm the view that the chief solids in the aqueous humour are metallic salts, protein being present only in traces. But the statement that the aqueous has a higher osmotic pressure than the blood is challenged. According to these results the formation of the aqueous is most easily explained by physical filtration.

As pointed out in the introduction there are certain problems presented by tissues in contact with a saline solution containing no protein or other colloid. Can it be that the endothelium of Descemet's membrane is actually water-proof, in which case the nutrition of the cornea would take place from the periphery? There are certain clinical and experimental facts which bear this out. "Absorption of fluid (by the cornea) from the aqueous occurs when the endothelium is injured by pathological processes, *e.g.* interstitial keratitis...Softening and ectasia of the cornea may ensue during life from prolonged impairment of the endothelium[1]."

Again, as is well known, loss of continuity of the anterior capsule of the lens will lead, in young subjects at least, to swelling and protrusion of the lens substance through the wound and ultimate breakdown of lens tissue. Does this capsule function in the same way as the endothelium of Descemet's membrane? Most interesting, too, are the problems concerned with the iris. We have here, what is not present in cornea or lens, a fairly rich blood supply. I do not desire to enter into the debateable question of intra-ocular pressure but one important point seems to me to have been overlooked. If in the capillaries and venules of the iris the pressure over and above the intraocular is greater than the osmotic pressure of the proteins of the blood then, according to all theory, there must be filtration outwards from the iridic vessels. But if the pressure over and above the intraocular is less than the osmotic pressure of the proteins of the blood then the aqueous humour will be absorbed. The argument is not affected even if the osmotic pressure of the humour is slightly higher than that of the blood. If **Parsons'**[1] estimates are correct, *viz.* that the intraocular pressure is 20 to 30 mm. Hg whilst the total capillary pressure is 40 to 50 mm. Hg then, as the proteins of the blood exert, according to **Starling**, an osmotic pressure of 30 mm. Hg, there must be a movement of aqueous humour into the iris capillaries with a head of pressure equal to 10 mm. Hg. As no one has measured the pressure in the iridic vessels this estimate must remain a surmise; but experiments with pigments have placed beyond doubt[2] that the iris, at least on its anterior aspect, does absorb fluid and so we may reason backwards that there the capillary pressure over and above the intraocular is less than 30 mm. Hg; also that no water-proof membrane envelopes the anterior surface of the iridic curtain—it is stated that there is no endothelium at this region.

---

[1] *The Pathology of the Eye*, by J. Herbert Parsons, p. 997.

If this reasoning is correct the iris should always be reckoned as a participant in the absorption of aqueous humour by virtue of the proteins of its blood. Diminished absorption would then be caused by pathological thickening of the vessel walls, high capillary pressure over and above the intraocular, or reduced iridic surface as occurs in mydriasis. Clinical evidence, I take it, is in favour of this view. Of course, such absorption could not explain the leakage that occurs in an excised eye under pressure; here one must fall back on the lymphatic path.

## RESULTS

(1) The osmotic pressure of ox aqueous humour was found to be equal to that of blood. Other quantitative estimations support the view that the humour is a blood filtrate.

(2) The osmotic pressure of the blood proteins in the iridic vessels will cause absorption of aqueous humour if the excess pressure in these vessels is less than 30 mm. Hg.

---

[1] *The Pathology of the Eye*, by **Herbert Parsons**, p. 1047.

[2] **J. Herbert Parsons**, *loc. cit.* p. 991.

# A SIMPLE METHOD OF SHORT-CIRCUITING THE PORTAL CIRCULATION. BY H. H. DALE AND P. P. LAIDLAW.

*(From the Department of Biochemistry and Pharmacology of the Medical Research Committee, and the Department of Pathology, Guy's Hospital.)*

IN some recent experiments on the mechanism of certain shock-like conditions we wished to observe the effect of excluding the liver alone from the circulation. The available methods for doing this were unsuitable for our purpose. Complications which we wished to avoid would be introduced by the use of hirudin and the exposure of the blood to injurious contact with artificial tubes. The making of Eck's fistula did not seem to be a practicable procedure in cats, on which most of our experiments were made; in any case it involves a relatively severe and prolonged operation, which was especially undesirable for our purpose. The simple procedure here described requires no special surgical dexterity, is easily carried out in the cat, and can be completed so rapidly that the animal is immediately in satisfactory condition for the main purpose of the experiment. It was demonstrated to the Physiological Society in October 1917.

The essential feature of the method is the use of an excised vein for making an anastomosis between the portal vein and the inferior cava. Apposition of endothelium to endothelium, so that the blood touches nothing but the uninjured lining of the veins, is secured by the use of Crile's anastomosis-cannulæ. The vein which we have used is the external jugular vein, which can be obtained from the same cat or from another. The only part of the procedure demanding time and patience is the attachment of a Crile's cannula to each end of this vein after its excision, and it is an advantage of the method that this can be done beforehand, without hurry, by using the vein from another cat.

The vein is exposed along its whole length in the neck and tied at each end. Small tributaries are tied or twisted and the vein is then dissected free and cut out. It is immediately transferred to a dish of solution containing 0·9 p.c. sodium chloride and 1 p.c. sodium citrate in distilled water.

In this all traces of blood are carefully washed from the interior of the vein. It is important at this stage not to lose the identification of the cephalic from the thoracic end of the vein, since valves are often present, and it is therefore essential that the flow of blood eventually established through it shall be in the normal direction.

The cannulæ should not be so large as to cause undue stretching of the vein wall in reflecting the cuff over the outside; on the other hand they must be large enough not greatly to restrict the lumen where the vein passes through the cannula. For cats we used small silver cannulæ intended for uniting artery to vein for direct transfusion in man, the internal diameters being about 1·5 to 2 mm. The method was used in dogs on a few occasions, and for these we turned some larger cannulæ (3 mm. diameter) out of short segments of an old silver catheter.

Each end of the vein in turn is drawn through a cannula of appropriate size and the cuff reflected and tied in position. It is desirable to use coloured silk for tying the cuff of the cephalic end, so that it can easily be recognised. The freedom of flow through the vein is tested by holding it up by the cephalic cannula and squirting a jet of saline into this end from a fine nozzle. If a shred of clot is detected at either orifice it can be removed with fine forceps, great care being taken not to touch the endothelial lining. The vein on its cannulæ is now laid in Ringer's solution until it is required. It is quite probable that it could be so kept on ice for some days, but we have not tested the point.

The animal in which the short circuit is to be made is now prepared. Under deep ether anæsthesia the abdomen is opened along the middle line, and the intestines packed aside with warm swabs. The systemic end of the anastomosis is first prepared. We have used either the right renal vein or the vena cava itself. In the former case the renal artery is first tied. The vein is tied close to the hilus of the kidney, a loose ligature is laid round it for tying in the end of the jugular vein, and a clamp is applied close to the vena cava. The kidney can then be removed, to give greater freedom of manipulation. If the anastomosis is made directly with the lower end of the vena cava, the aorta is tied below the origin of the renal arteries. The cava is then doubly ligatured above the entry of the iliac veins and cut between the ligatures. Lumbar branches are then tied and cut, so that the cava can be dissected free for two or three centimetres and turned forward. A loose ligature is laid ready and a clamp applied above it as in the case of the renal vein.

The portal vein is now prepared, being dissected clear from the point of entry of the splenic vein up to the portal fissures. One or two small tributaries from the pancreas and duodenum are double-ligatured and cut, so that the portal vein can be completely freed between the splenic vein and the liver. Two loose ligatures are then laid round it.

The renal vein or lower end of the cava, whichever has been chosen, is now incised distally to the clamp and the blood carefully washed out of the occluded segment with citrated saline. The *thoracic* end of the prepared jugular vein on its cannula is then inserted through the incision, pushed well into the lumen and tied in by means of the loose ligature laid ready. This systemic end of the anastomosis can be completed deliberately, but the insertion of the cephalic end of the jugular into the portal vein must be carried out with speed, since the portal vein must be clamped during its completion. Cooperation of two trained workers is useful at this stage, though not essential. The first holds the cephalic end of the jugular vein on its cannula ready for insertion and applies a clamp to the portal vein between the splenic vein and the lower ligature, while the second draws the upper ligature tight, close up to the portal fissure, incises the portal vein and holds the incision open. The first immediately inserts the end of the jugular vein on its cannula and the second ties it in position by means of the lower ligature, laid ready round the portal vein. The clamps on the portal and renal vein (or cava) are immediately removed, and the flow through the inserted jugular is at once established. It is advisable then to complete the cut through the portal vein above the anastomotic junction, so that it is no longer attached to the liver; any additional small dissection is made which may be necessary to allow the jugular vein and its junctions at both ends to be disposed without liability to kinking. The hepatic artery can then be tied or clamped, and the intestines carefully replaced, so as to avoid pressure on or twisting of the anastomotic path. The abdomen can then be closed.

The whole operation, from the first abdominal incision, should not take more than 15 to 20 minutes, and the clamp need not be on the portal vein more than about 20 seconds. If the arterial blood-pressure is recorded while the connexion is being established it will be seen to fall, perhaps, 60 mm. during the portal obstruction, but it quickly recovers when the anastomosis is completed and then maintains a good high level. A failure of recovery or renewed fall of the pressure probably indicates obstruction, the existence of which can be verified by the colour of the bowels. The only likely sources of this trouble are the formation of clot in the jugular vein at one end or the other, where its lumen is slightly constricted by passage through the cannulæ, or obstruction by kinking. The latter trouble is easily adjusted, but a clot denotes injury of the endothelium, and even if the obstruction caused in this way can be temporarily removed by manipulation, it is certain to recur, and it is better to abandon the experiment. We have not often met with this difficulty, and a little practice in the attachment of the vein to the cannula should eliminate it altogether. When once the circulation has been established and has remained satisfactory for ten minutes or so, it may be expected to continue so during the course of an ordinary experiment.

The method is quite as suitable for dogs as for cats.

Professor Bainbridge, who wished to observe the effect of temporarily diverting the blood from the liver, asked us to test the possibility of using the splenic instead of the main portal vein. We had no difficulty in doing this in a medium-sized dog. The splenic artery was tied and the cephalic end of the jugular was inserted into the splenic vein close to its opening into the portal, the thoracic end into the renal vein as usual. By clamping the interposed jugular, or the portal vein above the splenic orifice, the portal blood could be alternately sent through the liver or diverted into the vena cava.

It is possible that with absorbable cannulæ (say of thin ivory) and strictly aseptic procedure, the operation could be used for permanently short-circuiting the portal circulation. The renal vein would have to be used and one kidney removed. If the operation proved to be a practical alternative to the Eck fistula, the cat would become available for investigations of a kind hitherto restricted to dogs. We have not tested this possibility of making the connexion permanent. We suspect that kinking of the jugular vein would be liable to occur if its middle portion were left hanging free; probably it would be advisable to fix it to the back of the abdominal cavity by a few stitches through the adventitia of the vein and the parietal peritoneum.

# HISTAMINE SHOCK. By H. H. DALE and P. P. LAIDLAW.

*(From the Department of Biochemistry and Pharmacology of the Medical Research Committee, and the Department of Pathology, Guy's Hospital.)*

In our earlier papers(1, 2, 3) on the action of histamine we have been chiefly concerned with the physiological analysis of the evanescent effects of smaller doses, though mention was made in our first paper of the phenomena of fatal histamine-poisoning in rabbits and guinea-pigs and of the prolonged collapse and coma caused by injecting large doses into the unanæsthetised cat. The essential difference between its actions in rodents and carnivora is well illustrated by the modifications introduced by anæsthesia. The normal rabbit or guinea-pig is easily killed by a relatively small intravenous injection of histamine, but suffers little harm from a much larger injection when deeply under the influence of an anæsthetic. We have shown that the cause of death in the guinea-pig is bronchial constriction, in the rabbit obstruction of the pulmonary circulation leading to acute dilatation of the right ventricle, and have pointed out that the reaction of plain muscle to histamine, on which the effects depend, is weakened by an anæsthetic. The unanæsthetised cat, on the other hand, recovers from the depressant effects even of very large doses, while in the same species under an anæsthetic even moderate injections of histamine often produce a fatal circulatory collapse and respiratory failure, from which the animal does not recover even after prolonged application of artificial respiration. E. Mellanby(4) has studied the onset of this condition in anæsthetised cats as the result of absorption of histamine from the small intestine. Our object in this paper is to examine the nature and the mode of production of this "histamine-shock" in anæsthetised cats. The fact that the condition has many features in common with a number of pathological conditions, loosely classified as "shock," gives to its analysis a more than immediate interest.

*Methods.* Most of our experiments have been made on cats, a few only on dogs. It will be clear from what has been said above that the anæsthetic used has not merely the function of rendering the animal unconscious, but has some influence on the production of the effect we are studying. The precise nature of the adjuvant effect of the anæsthetic is not yet clear. It is not due to the presence of the anæsthetic as such, since a typical shock is produced by histamine in the decerebrated or spinal animal, after all traces of volatile anæsthetic have been removed. After recovery from a simple prolonged anæsthesia with ether, a cat remains for some time abnormally sensitive to the action of histamine, fatal shock being produced by a much smaller intravenous dose than that from which the normal unanæsthetised cat rapidly recovers.

Ether, following induction by chloroform, has been the anæsthetic in a large majority of the experiments. An animal under paraldehyde or urethane shows the effects quite typically, but in studying possibilities of resuscitation a volatile anæsthetic has the advantage that it can be lightened, discontinued and resumed in accordance with the need, as indicated by disappearance and revival of reflexes. The animals have, in most cases, been subjected to a minimum of operative preparation. A tracheal cannula was inserted to facilitate administration of the anæsthetic and to be ready for the application of artificial respiration at need. Cannulæ in one carotid artery and one femoral vein served for recording arterial pressure (mercury manometer) and making injections respectively. The other carotid artery was usually tied, clamped and cut to give samples of blood. Since failure of the vasomotor centre probably played some part in the later stages of the effect it was suspected that the restriction of its blood-supply by the ligature of both carotids might contribute to the result, when once the pressure had been reduced to a low level. The results were not perceptibly different, however, when one carotid was left patent and a femoral artery used for the collection of blood-samples. In a few experiments on the part played by the liver the circulation of this organ was short-circuited by a method which we have described in a recent note(5), the portal vein being connected to a renal vein or to the vena cava by means of an excised external jugular vein and Crile's anastomosis cannulæ. Throughout all experiments the animal was kept on an electrically heated table and an effort was made to eliminate any shock-producing factors other than those directly under examination.

The histamine used was the crystalline "Ergamine" phosphate of Burroughs Wellcome & Co. This is the diphosphate and contains roughly one-third of its weight of histamine base. Doses are expressed in milligrams of the base, the solution used being made up, as a rule, to contain

0·1 p.c. of this with 0·9 p.c. of sodium chloride. Solutions more than 24 hours old were not used; solutions of the phosphate, unless sterilised, rapidly lose their activity through the growth of bacteria in the solution.

*Sudden injection of a large dose.* When a dose of 1–2 mgm. of histamine per kilogram, dissolved in a few c.c., is rapidly injected into a vein, the action divides itself into three phases, two being brief preliminary effects, the third the main, lasting effect.

1. The immediate effect, usually beginning before the injection is completed, is a steep fall of the arterial pressure to the extent of 50 or 60 mm. This is undoubtedly due to lessened output from the left ventricle. **Dale** and **Laidlaw**(1) noticed this preliminary cardiac effect with an intermediate dose, showed that it was associated with shrinkage of a loop of bowel of which the volume was recorded, and attributed the effect to constriction of the pulmonary arterial branches. If the injection of a large dose is made into an animal with the chest opened, under artificial respiration, the distension of the right side of the heart during this initial fall of blood-pressure is very clearly apparent. In a record taken with Cushny's myocardiograph the contractions of the right ventricle are seen to become weakened and irregular during the period of overdistension. In one experiment death occurred at this stage from ventricular fibrillation. In an experiment on the heart-lung preparation from a dog **Fühner** and **Starling**(6) observed a rise of pulmonary pressure on adding histamine to the circulating blood. This was accompanied by a rise of pressure in the left auricle, which, since the arterial resistance was artificial and constant, would seem to indicate a direct weakening of the action of the left ventricle. We are not in a position definitely to exclude such a direct weakening of the heart's action in the cat, though the fact that the isolated, perfused heart is stimulated by histamine to more rapid and ample contractions is against it. In any case we are convinced that this initial fall of arterial pressure is, in the main, the result of pulmonary arterial constriction. It is, therefore, an entirely different phenomenon from the rapid, purely vasodilator fall produced by a very small dose of histamine. It lasts only a few seconds; the distended right ventricle almost suddenly empties itself and resumes its normal, unembarrassed activity.

2. The second phase, beginning with the recovery of the heart from the first effect, sometimes appears merely as a brief arrest of the downward tendency of the arterial pressure, but frequently as a distinct secondary rise of pressure, which may even surpass the original level. If the vagi are intact this is obscured by a pronounced slowing of the

heart-beat, obviously due to action on the medullary centre, since it is abolished by section of the nerves. We usually eliminated this by cutting the vagi before the injection was given. This secondary phase of delay, partial recovery, or actual rise of arterial pressure, we regard as due to the arterio-constrictor effect, which histamine in large doses produces in virtue of its stimulant action on plain muscle. In the rabbit this is often its main effect on the circulation. In a cat in which a very low state of arterial tone has been produced, as by complete pithing, the immediate effect of injecting a large dose (1 mgm. or more) of histamine is to cause a rise of blood-pressure. A similar brief pressor effect follows injection of a second dose of histamine during the "shock" produced by the first dose. When it appears as the second phase of the effect of histamine, injected when the initial blood-pressure is high, this pressor effect only lasts from 10 to 30 seconds.

Fig. 1. Cat. 2·350 kilo.

3. As the second phase of recovery or delay passes off the main fall of pressure begins. This is slow in comparison with the initial steep fall, and as it progresses the pulse-waves recorded by the manometer become rapidly smaller, until by the time the blood-pressure has reached its minimum (usually about 30–50 mm.), which is attained in about 4 to 10 minutes after the injection, the pulse is often hardly perceptible on

the tracing. Soon after the onset of the main fall of pressure the respiration becomes deep and laboured, then slower, and as a rule progressively weaker, till it ceases altogether and artificial respiration becomes necessary. Occasionally infrequent inspiratory gasps persist after the artificial respiration has begun. The application of artificial respiration does not arrest the fall of pressure; more often it seems to accelerate it. When the effect has become fully developed the manometer float draws on the drum an almost level line, scarcely broken by the tiny undulations caused by the heart-beats and the artificial respiration. The different early phases of the effect on the blood-pressure are illustrated in Figs. 1–4. Fig. 1 shows in characteristic form the primary fall, secondary recovery, and third main fall of pressure, the heart-beats being no longer

Fig. 2. Cat. 3·0 kilo.

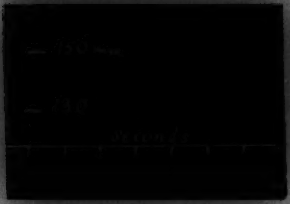


Fig. 3. Cat. 2·5 kilo. Normal.

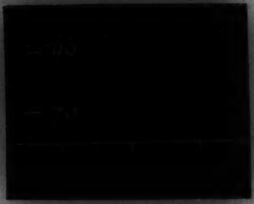


Fig. 4. The same, 10 minutes after injection of 5 mgm. of histamine.

perceptible on the record when the pressure has fallen to about 60 mm. Fig. 2 shows an example of less extreme effect, in which the respiration persists longer than usual. The secondary vaso-constrictor effect carries the pressure well above the original level in this instance. Figs. 3 and 4 show portions of another record, taken with a faster drum. Fig. 3 shows the arterial manometer record before, Fig. 4 the same 10 minutes after injection of 5 mgm. of histamine. It will be seen that the pulse transmitted to the manometer has already become extremely small when the pressure is still at about 80 mm. Figs. 5–7 illustrate the effect on a dog.

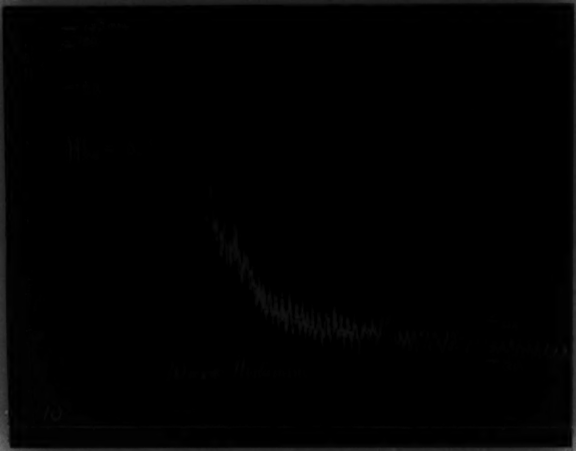


Fig. 5. Dog. 11 kilo.

The secondary rise of pressure is not seen in this animal, and the earlier stages of the fall are accompanied by pronounced dyspnœa. Otherwise the general features of the action are similar to those seen in the cat.

When the effect is fully developed the animal lies relaxed and apathetic, the corneal reflex is abolished, and the volatile anæsthetic can be discontinued. The mucous membranes show a blueish pallor. Unless the animal is artificially warmed the body temperature falls with great rapidity.

*Slow infusion of histamine.* The effect of a large dose of histamine suddenly injected, as described above, presents a curious contrast with that of a small dose, as described in our earlier papers. The intense sudden vasodilator action of the small dose seemed to be so complicated by the concomitant vaso-constrictor action in the case of the larger dose, that the whole effect appeared, at first sight, to be of a different type. It was of interest to enquire, therefore, what would be the effect of the larger dose when slowly infused in more dilute solution, so that the whole injection occupied about 20 minutes instead of about 20 seconds. The solution was made up to contain 0·2 mgm. of histamine base per c.c. of physiological saline and, by means of a device for securing a slow, steady infusion, was run into the femoral vein at the rate of about 1 c.c. in 2 minutes, so that 0·1 mgm. entered the circulation in each minute, or 0·01 mgm. in 6 seconds. The onset of the infusion was followed by a prompt fall of blood-pressure from 160 mm. to 80 mm., such as would follow the injection of 0·01 mgm. The pressure, however, under the continued infusion, did not recover, but remained fairly steady at about 80–70 mm. until 0·6 mgm. in all had been injected. Then, with continued infusion, the output of the heart, as indicated by the pulse waves recorded by the manometer, began to decline and the pressure to fall further; meanwhile the respiration gradually became slower and weaker and then failed altogether. Artificial respiration was applied, but the pressure still declined and had fallen to 46 mm. by the time the injection of 1 mgm. was completed (13 minutes from the start). Thereafter the pressure slowly rose to 56 mm. with continued infusion. The natural respiratory movements were at this stage represented only by infrequent

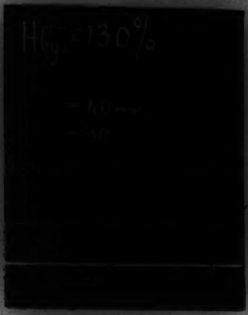


Fig. 6. Continuation of Fig. 5, 7 minutes after the injection.

Fig. 7. The same 11 minutes after the injection.

convulsive inspirations, which appeared to be efforts at vomiting. By the time a total of 2 mgm. had been injected (representing a little more than 1 mgm. per kilo., the cat weighing 1·8 kilo.) the heart-beats were scarcely perceptible on the pressure tracing, while the pressure had returned to 46 mm. and continued to decline to 36 mm. after the injection had been completed. Portions of this record are reproduced in Fig. 8. It will be seen that the result of prolonging the time of injection, so that the amount of histamine circulating at any moment was probably always small, was to eliminate the first two phases of the effect of a sudden large injection, which we attributed to constriction of the pulmonary and systemic arteries, leaving only the shock-like third phase, with a more gradual and insidious onset. The connexion of this phase with the vasodilator effect of the single small dose is thus rendered obvious. When the vasodilator effect is perpetuated by continued slow infusion of the drug the shock-like failure of the circulation soon appears.

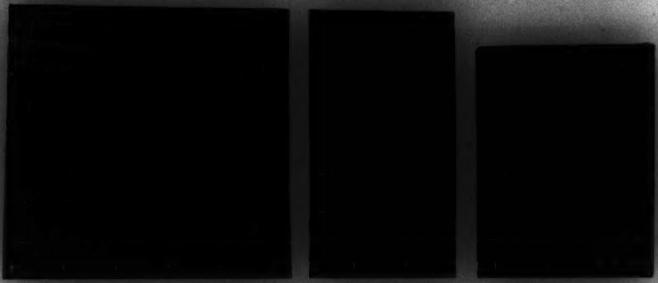


Fig. 8. Cat 1·8 kilo. At *A* begin infusion of 0·02 p.c. histamine. At *B* 1 c.c. in. At *C*, 13 minutes from the start, 5 c.c. = 1 mgm. in. At *D*, 25 minutes from the start, 10 c.c. = 2 mgm. in. Infusion stopped.

## ANALYSIS OF THE "SHOCK."

1. *The action on the heart.* A glance at the manometer record is sufficient to indicate diminution, almost to the point of extinction, of the output of the heart as a principal factor, if not the sole cause of the circulatory collapse. A casual inspection of such a record might easily suggest that we are dealing with a progressive primary failure of the heart's action. It soon becomes clear, however, that such an interpretation is incorrect. If the cardiac contractions had become so enfeebled

as the record at first sight suggests, we should expect the blood-pressure to have fallen to 20 mm. or less, whereas it often remains at 40, 50 or even as high as 70 mm. If the ear is applied to the chest wall the heart-sounds are well audible, even though the excursions of the mercury in the arterial manometer may be hardly sufficient to move the recording float. By lightly grasping the chest wall a relatively strong cardiac impulse can be detected. These auditory and tactile impressions of well-maintained muscular activity of the heart receive visual confirmation when the chest wall is opened. There is usually a slight excess of pericardial fluid, which somewhat obscures the movements of the heart until the pericardium is opened. This, however, is not the cause of the low blood-pressure and small pulse, since neither is improved by opening the sac. When the heart is exposed it is seen to be beating with surprising vigour. The impression given by simple inspection is confirmed by a mechanical record of the contractions with the myocardiograph, as shown in Fig. 9. In this record the excursions of the lever recording the contractions of the right ventricle measure 25–29 mm. when the blood-pressure stands at about 90 mm., before the injection of histamine; 6 minutes after the injection, when the preliminary fall, due to cardiac embarrassment, and the secondary vaso-constrictor rise have given way to the main permanent fall of pressure, the lever excursions measure 32 mm., though the pressure has sunk to 44 mm., and still measure 26 mm. when the blood-pressure has reached 26 mm., 18 minutes after the injection.

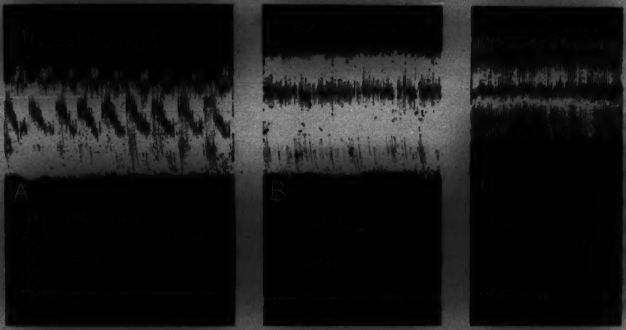


Fig. 9. Cat. 2·6 kilo. *A* normal, *B* 6 minutes, *C* 18 minutes after injection of 5 mgm. of histamine.

Clearly the reduced output, so evident on the manometer record, is not due to weakening of the muscular contractions of the ventricles. When the circulation is failing from primary cardiac weakness the feebly beating heart is distended with blood, especially its right chambers. The heart of the cat in histamine shock is beating strongly, but the beats are ineffective because the heart is practically empty. The wall of the right ventricle is flaccid and folded in diastole; if the ventricles are squeezed between the fingers the effect on the arterial manometer is hardly perceptible; if a cut is made through the wall of either ventricle only a few drops of blood are expelled through the incision at each systole, and the outflow ceases altogether by the time some 10 c.c. have been ejected. The systolic output from the heart is failing because the diastolic filling is inadequate.

This failure of the heart to fill itself is, again, not due to defective diastolic relaxation. We are not able to exclude a slight increase in the tone, and consequent slightly diminished relaxation of the heart in diastole, as a possible direct effect of histamine on the heart muscle. Mere inspection of the beating heart easily gives the impression of increased tonus, since the eye cannot readily distinguish between defective relaxation of the muscle and inadequate filling from the veins. Several observers have stated that the tonus of the heart muscle is increased in surgical shock. It is quite clear, however, that imperfect relaxation is not to any significant degree responsible for the deficient filling of the heart in histamine shock, for the intrathoracic venæ cavæ are not distended, but flaccid and unusually empty. If 20 c.c. or so of warm saline solution are run into a jugular vein it can be seen that the chambers of the heart fill readily in diastole, and the filling is accompanied by a rapid recovery of the output and a rise of arterial pressure. The restorative effect of such a saline injection is but temporary, for reasons to be discussed later, and can be repeated many times. We may safely conclude that the failing output is due to some extracardiac condition affecting the venous return.

2. *The distribution of blood in the peripheral vessels.* We have already seen that the blood is not in the heart or in the venæ cavæ in the thorax. Plethysmographic records of the volume of organs give us little additional information in this case. A loop of bowel and a limb, after brief shrinkage during the initial fall and recovery of blood-pressure, both show a steady, slow increase of volume with the secondary, lasting decline of pressure. This, however, merely shows that the accumulation of blood at the periphery, which reasoning by process of exclusion shows to be occurring,

is not restricted to a particular area, but is taking place in abdominal viscera and skeletal muscles alike. Experience of the effect of other poisons having a generally similar type of action, as, for instance, of the effect of peptone in the dog, would suggest the probability of an accumulation of blood in the liver. Simple inspection of the exposed organ is sufficient to show that histamine does not produce this effect in the cat. The liver is not abnormally dark or swollen, and if incised it bleeds but little. The portal vein and its main tributaries are flat and collapsed, being even more conspicuously empty than the vena cava. If the lungs are allowed to collapse, by stopping the artificial respiration after the chest has been opened, it is obvious that they also are not to any significant degree congested with blood.

The blood, then, is not accumulated in the heart, or the great veins, which, on the contrary, are abnormally empty, or in the liver or the lungs, which are certainly not congested. Inspection further shows that the aorta, the main arteries, and even the finest arterial branches which can be distinguished on the surface of the intestine, contain very little blood. We have not investigated, by the method of comparative rates of perfusion, whether the arteries at this stage of the action are actively constricted. The fact that a further injection of histamine will produce a rise of pressure indicates that the initial vaso-constrictor effect has at least to some extent passed off. If there is still some constriction, this is not the reason for the small blood content, for the larger arteries are obviously flat and collapsed. The point of main interest for our purpose was that no part of the missing blood could be accounted for by accumulation on the arterial side of the circulation.

There remained the capillaries and venules, and here, at last, signs of accumulation of blood could be detected. The signs were clearest in the case of the abdominal viscera, possibly on account of the greater transparency of the tissues. If the abdomen was opened in the earlier stages of the main, secondary fall of the blood-pressure, the intestines looked diffusely and somewhat duskily red and the net-work of venules stood out distinctly. The pancreas had a purplish, congested appearance, which was seen in less degree in the lymphatic glands. At a later stage the general colour of the intestines was much bluer, while the pancreas became very obviously œdematous, the lobules appearing separated, as if embedded in a clear and colourless jelly. No other organ showed this pronounced œdema, nor was there any distinct accumulation of free fluid in the serous cavities, though the surface of the viscera was unusually moist. Several attempts were made to observe changes in the calibre of capillaries under a moderate power of the microscope, without any result to which we could attribute importance. When the shock effect was well developed, the extreme slowness of circulation, amounting locally to practical stagnation of the blood, was observed in the microscopic venules. In the vessels where the flow was extremely sluggish the column of blood seemed often to be divided in segments, clear spaces, apparently only containing plasma, intervening between short columns of tightly packed corpuscles. There was no pronounced agglutination of the corpuscles, however, in blood removed from the carotid artery.

We have spoken of the great veins and their main tributaries as very empty and collapsed and of the capillaries and minute veins as full. The veins of intermediate size, such as the branches just leaving the wall of the bowel to join the veins in the mesentery, often showed a curious condition which deserves mention. They appeared to be irregularly constricted, so that a short stretch of vein, 2–5 mm. in length, distended to sausage-shape with blood, would intervene between sections in which the vein was reduced to a thread-like slenderness. It must be supposed that irregularly distributed tone of the plain muscle in the vein walls produced this condition. It should be noted, however, that it only appeared at a late stage of the action, when the circulation through the bowels was already extremely slow. It cannot, therefore, be regarded as pointing to constriction of the smaller veins as a factor in the production of the failure of the circulation.

We have examined the other organs of the body, and especially the skeletal muscles, as containing a large part of the whole capillary area, for signs of an accumulation of blood in the capillaries, similar to that which we detected in the walls of the bowel and in the pancreas. In the straightforward experiments we were unable to satisfy ourselves on this point, but in experiments where a state of plethora had been previously produced by transfusion of whole blood from another cat the general peripheral engorgement produced by histamine was very striking. In two experiments of this type anastomosis was made between the jugular veins of two cats under ether by means of Crile's cannula, and it was arranged that the larger animal in each case should serve as the donor to the smaller, which was the experimental animal. Transfusion before the administration of histamine did not materially alter the response in the experimental animal. In one experiment profound shock resulted and a further transfusion did not bring about any permanent improvement. *Post mortem* the congestion of the visceral capillaries was very remarkable and the exsanguinated cat alongside made a very vivid contrast.

The colour and general appearance of the intestines resembled that met with in early strangulation of the bowel, yet the veins were not distended. The liver was of a dark plum-colour, but not obviously enlarged, and the skeletal muscles were deep red and obviously contained much blood. Unfortunately the corpuscular content of the blood was not determined before administering histamine, but it cannot be doubted that a pronounced concentration occurred, since the subsequent hæmoglobin reading was 140 p.c. and the count of the red cells 13,250,000 per cmm. This evidence that part of the missing blood collects in the capillaries of the voluntary muscles is confirmed by the observation, to be described later, that the condition of shock can be produced by histamine after removal of the abdominal viscera. Mention may here be made of an attempt to measure the distribution of blood between the abdominal viscera and the rest of the body.

A cat was anæsthetised with ether, and a sample of blood taken from a carotid artery. The abdomen was then opened in the middle line, the rectum was double ligatured and cut through as low down as possible, and the œsophagus treated similarly just below the diaphragm. Double ligatures were then laid round (1) the inferior mesenteric artery, (2) the superior mesenteric artery and the cœliac axis close to their origin from the aorta, (3) the portal vein above the points of entry of the splenic and pancreaticoduodenal veins, and (4) the vena cava just below the liver. A single ligature was passed round the vena cava between the liver and the diaphragm. When all was ready one of the ligatures on each of the arteries to the intestine and stomach and on the portal vein was drawn tight, the cooperation of three persons, working to a signal, ensuring simultaneous occlusion of all. Without delay the ligatures on the vena cava above and below the liver were then tied, the chest was immediately opened, the heart was ligatured, and the inferior vena cava tied above the diaphragm. We now had the animal dead, but had shut into (1) the vessels of the stomach, intestine and spleen, (2) those of the liver, and (3) the rest of the body, the amounts of blood which they respectively contained during life, under the conditions of circulation existing at the moment when the ligatures were tied. Not much stress could be laid on the blood content of the liver, since the portal vein was tied some seconds before the vena cava. Our main object however—a determination of the fractions of the total blood in the intestines and in the rest of the body—could now be attained. The arteries were ligatured again and cut between the ligatures. The portal vein also was again ligatured close to the portal fissure and cut through. The stomach, intestines and spleen could now be removed entire, without loss of blood, to a large dish. Cannulæ were inserted into the arteries and infusion was carried out through each in turn with oxalated saline solution, the portal vein being slit open to allow free outflow. The washing was continued till no blood could be detected in any vessels and the outflowing fluid was colourless. The mixed washings were carefully collected and made up to an exact volume. The liver was next removed, with the closed section of the vena cava attached to it, and was similarly washed out completely through the portal vein; periodical occlusion of the outflow through the vena cava, causing temporary distension of the liver, greatly assisted the complete exsanguination. Finally the blood in the rest of the body was washed out, infusion being made into the root of the aorta, with outflow cannulæ in the abdominal vena cava and the right auricle, and into the pulmonary artery, with an outflow cannula in the left auricle.

The blood from the ventricles was washed out and added. We had thus three measured quantities of saline containing the blood (1) in the stomach, intestines and spleen, (2) in the liver, and (3) in the rest of the body, arrested at a moment of normal circulation. The corpuscles in each were completely hæmolysed by adding ether and shaking, and the solutions were then freed from suspended traces of cellular debris by centrifuging. The volume of blood in each was then estimated by colorimetric determination as CO-hæmoglobin against an accurate dilution of the original blood. A precisely similar determination was then carried out on another cat, in which the ligatures on the vessels were tied at the height of the shock produced by histamine. The results were as follows:

1. Normal cat. Weight 2·3 kilo. Calculated blood volume (taken as 48 c.c. per kilo.) =110 c.c.

The following dilutions gave equal depths of colour.

Normal carotid blood × 82.
Washings from liver (800 c.c.) × $\frac{58}{50}$.
Washings from stomach, intestine and spleen (500 c.c.) × $\frac{96}{50}$.
Washings from remainder (2000 c.c.) × $\frac{156}{50}$.

Liver blood $= \frac{800 \times 58}{50 \times 82}$ c.c. = 11·3 c.c. = 10·3 p.c.

Stomach, intestine and spleen blood $= \frac{500 \times 96}{50 \times 82}$ c.c. = 11·7 c.c. = 10·6

Blood from remainder $= \frac{2000 \times 156}{50 \times 82}$ c.c. = 76·1 c.c. = 69·2

Total recovered = 99·1 c.c. = 90·1

2. Cat under histamine shock. Weight 2·35 kilo. Calculated original blood volume =113 c.c.

The following dilutions gave equal depth of colour:

Carotid blood (before histamine) × 72.
Liver washings (700 c.c.) × $\frac{62}{50}$.
Washings from stomach, intestine and spleen (500 c.c.) × $\frac{82}{50}$.
Washings from remainder (2500 c.c.) × $\frac{93}{50}$.

| | |
|---|---|
| Liver blood | = 12·0 c.c. = 10·6 p.c. |
| Stomach, intestine and spleen blood | = 11·4 c.c. = 10·1 |
| Blood from remainder | = 64·6 c.c. = 57·2 |
| Total recovered | = 88·0 c.c. = 77·9 |

It is difficult to draw from these figures a definite conclusion as to a change in distribution of the blood, as between the abdominal viscera and the rest of the body, in histamine shock. Of the blood removable by washing out the vessels with saline, that from the abdominal viscera bore a higher proportion to that from the rest of the body in the case of histamine shock than in the normal control. On the other hand the blood from the abdominal viscera in histamine shock does not form an abnormally large fraction of the original total volume of blood in the body, as calculated from the body weight. Its apparent increase in proportion to the blood from the rest of the body is due to reduction of the latter; the total amount obtainable by washing being only about 78 p.c. of the calculated volume, as compared with 90 p.c. in the normal animal. This suggests that there are probably capillary areas in which the corpuscles have become adherent, or condensed into solid columns, so that washing does not remove them.

An experiment of a somewhat different kind was made to determine the distribution of blood between large vessels, on the one hand, and small vessels and capillaries on the other.

In a cat under ether the abdomen was opened, the small intestine was cut through at two points, and the separated loop washed out thoroughly and tied at each end. The mesentery was then slit down to the root opposite each cut. Two ligatures were laid round the vessels of this isolated triangular segment of mesentery as near to the root as possible. The mesentery with the attached segment of intestine was then carefully spread out on a glass plate covered with thin rubber membrane. The preparation was carefully kept warm and moist by swabs of warm saline. At a given moment one observer tied the vessels of the segment of mesentery near the root, while the other occluded them close to the intestinal wall by pressure between the edge of a circular glass Petri-dish of suitable size and the rubber-covered plate. The second ligature near the root of the mesentery was then tied, and by cutting between the ligatures the preparation was freed and could be lifted from the body while the peripheral occlusion was maintained. With a sharp knife the loop of intestine was then separated by a cut peripheral to the compression and dropped immediately into a vessel containing oxalated saline solution. The pressure was then released and the wedge of mesentery with its vessels, together with blood which exuded from them on to the plate, was washed into a separate similar vessel. The loop of intestine was slit open and thoroughly squeezed and kneaded in the saline till it looked quite white. It was then removed, minced and ground with sand and washed with further saline, but as these washings were practically colourless they were discarded. The mesenteric vessels were squeezed thoroughly free from blood in the other dish. The two sets of washings were then made up to exact volumes, hæmolysed with ether, centrifuged and compared colorimetrically with a dilution of blood from the carotid artery in distilled water.

Arrangements being made to record blood-pressure and give intravenous injections, the cat now received 5 mgm. of histamine intravenously. (The body weight being 2·8 kilo. this amounted to about 1·8 mgm. per kilogram.) When the shock had completely developed, an adjoining, shorter loop of small intestine was treated in exactly the same way as the first. It will be shown later that the proportion of corpuscles to plasma is greatly increased in histamine shock. The determinations, however, were all made with reference to dilutions of the original blood.

The following were the figures obtained:

1. Before histamine.

Washings from intestinal wall—26 c.c. diluted $\frac{81}{50}$ matched carotid blood diluted 128-fold

$$\frac{26 \times 81}{50 \times 128} = 0{\cdot}33 \text{ c.c.}$$

Washings from mesenteric vessels—22 c.c. diluted $\frac{115}{50}$ matched carotid blood diluted 64-fold

$$\frac{22 \times 115}{50 \times 64} = 0{\cdot}79 \text{ c.c.}$$

2. During histamine shock.

Washings from intestinal wall—43 c.c. diluted $\frac{65}{50}$ matched carotid blood diluted 128-fold

$$\frac{43 \times 65}{50 \times 128} = 0{\cdot}44 \text{ c.c.}$$

Washings from mesenteric vessels—33 c.c. diluted $\frac{64}{50}$ matched carotid blood diluted 128-fold

$$\frac{33 \times 64}{50 \times 128} = 0{\cdot}33 \text{ c.c.}$$

The ratios $\frac{\text{blood in large vessels}}{\text{blood in small branches and capillaries}}$ are, therefore, in the two cases:

$$\text{normal} \quad \ldots \quad -\frac{0{\cdot}79}{0{\cdot}33} = 2{\cdot}4,$$

$$\text{histamine shock} - \frac{0{\cdot}33}{0{\cdot}44} = 0{\cdot}75.$$

These measurements confirm the visual impression that an essential factor in the failure of circulation, which we have called "histamine shock," is an accumulation of the blood in the minute vessels at the periphery, so that the larger vessels, both arteries and veins, are depleted, and the output from the heart falls to a very low level, owing to a gross deficiency in the venous inflow. The measurements do not indicate that this peripheral accumulation is restricted to any particular part of the system; the evidence points rather to its occurrence in all organs of the body alike. We shall see later that this is confirmed in another way.

3. *Changes in the blood.* (*a*) *Estimates of corpuscular content.* If a small sample of blood be taken from the carotid artery before the injection of histamine, and another when the "shock" has become thoroughly developed, a striking increase in the corpuscular content is usually found. This can be detected either by the hæmatocrit, by the hæmoglobinometer, or by counting the cells with a hæmocytometer. Having satisfied ourselves that these different methods gave parallel results, we adopted the hæmoglobinometer (Gowers-Haldane) for routine use, sometimes checking the results by use of the hæmatocrit. The samples were taken into small tubes containing a pinch of dry, finely powdered potassium oxalate, and all determinations of corpuscular content made at the end of the experiment.

The following record from an actual experiment illustrates the procedure and the type of result observed:

Cat weighing 2·35 kilo., anæsthetised with chloroform followed by pure ether. Right carotid dissected for taking blood samples; cannula in left carotid for recording arterial pressure. Injections into femoral vein.

3.26 p.m.—arterial pressure 175 mm. Blood sample No. 1.
3.32 p.m. „ „ 173 mm. Inject 5 mgm. of histamine.
3.38 p.m. „ „ 40 mm. Blood sample No. 2.
3.42 p.m. Animal killed for examination of tissues.

| | Hæmoglobinometer | Hæmatocrit |
|---|---|---|
| Sample No. 1 | 72 % | 30 % |
| „ 2 | 120 | 50 |

It will be noticed that the proportion between the two readings is the same by either method, being 3 : 5.

In other experiments, in which a similar comparison was made, the following values were obtained:

Table I.

| | Hæmoglobinometer | | | Hæmatocrit | | |
|---|---|---|---|---|---|---|
| | Normal | Shock | Ratio | Normal | Shock | Ratio |
| 1. | 78 | 116 | 1·49 | 33 | 50 | 1·5 |
| 2. | 80 | 120 | 1·5 | 33 | 50 | 1·5 |
| 3. | 92 | 130 | 1·4 | 33 | 50 | 1·5 |
| 4. | 80 | 118 | 1·3 | 40 | 52 | 1·3 |

It will be seen that, within the limits of accuracy of the methods, the proportions between the readings before and after histamine correspond. It will be seen, also, that the normal corpuscular content of the cat's blood does not often vary very much from about 33 p.c. by volume, while the hæmoglobin value varies more widely. Without any serious error, therefore, we can deduce hæmatocrit readings from hæmoglobinometer readings, in cases where only the latter were made, by assuming that the initial, normal hæmoglobin-reading corresponded to a corpuscle-content of 33 p.c., and that the change in relative corpuscular volume was proportional to the change in hæmoglobin value. The following table shows the arterial pressure and the hæmoglobin readings from arterial blood before the injection of histamine and at the height of the histamine shock in a number of cats, including those quoted above.

Table II.

| Blood-pressure in mm. of mercury | | Hæmoglobin % | |
|---|---|---|---|
| Normal | Histamine shock | Normal | Histamine shock |
| 175 | 40 | 72 | 120 |
| 173 | 40 | 92 | 130 |
| 143 | 70 | 80 | 98 |
| 206 | 46 | 96 | 142 |
| 188 | 64 | 96 | 148 |
| 150 | 34 | 70 | 100 |
| 160 | 62 | 70 | 110 |
| 218 | 76 | 82 | 106 |
| 188 | 77 | 82 | 108 |
| 139 | 40 | 70 | 122 |
| 190 | 50 | 80 | 106 |
| 140 | 45 | 78 | 115 |
| 150 | 60 | 64 | 96 |

In all these cases the increase in hæmoglobin value is pronounced, though its extent is somewhat variable.

A single example will suffice to demonstrate the parallelism between the change in hæmoglobin value, corpuscular volume by hæmatocrit, and red-cell count.

| Hæmoglobinometer | | | Hæmatocrit | | | Red cells per c. mm. | | |
|---|---|---|---|---|---|---|---|---|
| Normal | Shock | Ratio | Normal | Shock | Ratio | Normal | Shock | Ratio |
| 74 | 98 | 1·32 | 30 | 40 | 1·33 | 8,344,000 | 11,360,000 | 1·36 |

When we consider the methods by which a rapid change of this nature could be produced, only two possibilities present themselves—loss of fluid from the blood vessels, or the washing into circulation of accumulations of corpuscles from some depot where they normally lie stationary. The close correspondence between the hæmoglobin and hæmatocrit readings is, on the whole, not in favour of the latter explanation; it might be expected, perhaps, that corpuscles thus freshly added to the circulation would have a perceptibly different hæmoglobin index from that of those normally circulating. The argument is not a very strong one, however, and must have less weight than the difficulty due to the consideration, that the production of the change in this way would necessitate the presence in the body, but not in circulation, of at least half as many corpuscles as are normally circulating.

There is no such difficulty in supposing that the increase in the proportion of corpuscles is due to passage of fluid from the blood into the tissues. Evidence of such passage is to be found in the accelerated flow of lymph from the thoracic duct, under the influence of histamine, as described in an earlier paper (Dale and Laidlaw(2)).

(*b*) *Direct measurement of plasma volume*. This supposition, that the increase in corpuscular content was wholly due to loss of fluid from the blood, was directly confirmed by the use of "vital red," introduced by Keith, Rowntree and Geraghty(7). "Vital red" is the name given by these authors to a complex dye, which they found to be physiologically very inert, to have practically no tendency to stain the tissues, and to be excreted very slowly. If a small dose of this substance, in watery solution, is injected into the circulation, its dilution in the plasma can be colorimetrically estimated in a sample taken a few minutes later. An equal amount of the normal plasma is added to the standard dilution of the dye which is used for comparison. After an interval a further injection of the dye can be given and the measurement repeated, a sample of plasma taken just before this second injection being, in this case, added to the control dilution. One such experiment may be described in detail, as it illustrates well the concordance of the results obtained by the different methods.

*Cat weighing* 2·3 kilo. Anæsthetised with chloroform, followed by ether. Arterial pressure from the left, samples of blood from the right carotid artery. Vagi cut.

3.25 p.m. Blood-pressure 170 mm. Bled 5 c.c. for control plasma (S 1).
3.29 „ B.P. 156 mm. Inject 0·8 c.c. of 1 p.c. vital red into femoral vein.
3.33 „ B.P. 136 mm. Bled 5 c.c. for colorimetric estimation (S 2). B.P. sinks to 110 mm. Lighten ether.
3.54 „ B.P. steady at 124 mm.
3.56 „ Inject 3 mgm. of histamine into femoral vein. B.P. falls rapidly to 96, rises again to 120, and then falls steadily, with slowing of respiration.
3.59 „ B.P. 55 mm. Respiration fails. Artificial respiration applied. Corneal reflex absent. Ether discontinued.
4.0 „ B.P. 40 mm.
4.0 to 4.3 p.m. Bled 4 c.c. (very slow flow from carotid) for second control plasma (S 3).
4.4 „ B.P. 34 mm. Inject 0·8 c.c. vital red into femoral vein.
4.6 „ Infrequent, inefficient inspiratory spasms. Artificial respiration continued.
4.8 „ B.P. 37 mm. Bled 5 c.c. for second colorimetric estimation (S 4). B.P. sinks to 26 mm.
4.10 „ Abdomen and thorax opened. Bowels show general purplish flush. Venules distinct, with very dark blood. Portal vein collapsed, with very little blood. Vena cava inferior not well filled. Heart beating vigorously, but chambers almost empty. Pancreas dusky in colour and very œdematous. Cat killed.

*Estimations on blood.*

*Colorimetric.*

1. *Normal*—Standard dilution—1 part plasma S 1, 2 parts vital red, 1 p.c. × $\frac{1}{200}$, 1 part saline.

Test solution—1 part plasma S 2, 3 parts saline.

Reading with Dubosq colorimeter—Standard 10, Test 10·9.

Then if $V_1$ = the volume of plasma in the cat,

$$\frac{0{\cdot}8}{V_1} \times \frac{1}{4} \times 10{\cdot}9 = \frac{1}{200} \times \frac{1}{2} \times 10.$$

$$\therefore\ V_1 = 0{\cdot}8 \times 109 = 87{\cdot}2 \text{ c.c.}$$

2. *During histamine shock.*

Standard dilution—3 parts plasma S 3, 8 parts vital red, 1 p.c. × $\frac{1}{200}$, 1 part saline.

Test solution— 1 part plasma S 4, 3 parts saline.

Reading with colorimeter—Standard 10, Test 8·5.

Then if $V_2$ = the volume of the plasma during shock,

$$\frac{0{\cdot}8}{V_2} \times \frac{1}{4} \times 8{\cdot}5 = \frac{1}{200} \times \frac{8}{12} \times 10.$$

$$\therefore\ V_2 = 6 \times 8{\cdot}5 = 51{\cdot}0 \text{ c.c.}$$

| | | |
|---|---|---|
| *Hæmatocrit* | Normal | 15·5 in 38·5 = 40 p.c. corpuscles, 60 p.c. plasma. |
| | Shock | 21 in 40·5 = 52 p.c. corpuscles, 48 p.c. plasma. |
| *Blood volumes* | Normal | $87{\cdot}2 \times \frac{100}{60} = 145{\cdot}3$ c.c.; Shock $51 \times \frac{100}{48} = 106$ c.c. |
| *Hæmoglobinometer* | Normal | 90. |
| | Shock | 118. |

From $V_1$ and the normal hæmatocrit reading we can calculate the original volume of corpuscles, $87{\cdot}2 \times \frac{4}{6} = 58{\cdot}1$ c.c. This has been reduced, however, before the second estimation by two bleedings of 5 and 4 c.c. respectively, of which the first contained $\frac{4}{10} \times 5 = 2$ c.c., and the second $\frac{52}{100} \times 4 = 2{\cdot}1$ c.c.; total $= 4{\cdot}1$ c.c. of corpuscles. The corpuscle-volume at the time of the second injection of vital red may be taken as $58{\cdot}1 - 4{\cdot}1 = 54$ c.c. (The direct determination above gives $106 - 51 = 55$ c.c.) From this the second plasma volume should be $54 \times \frac{48}{52} = 49{\cdot}8$ c.c., which corresponds as closely as can be expected with the 51 c.c. determined by vital red. We shall see later that under different conditions the loss of volume determined by vital red may be far greater than that indicated by the change in corpuscular content. The hæmoglobinometer readings as usual correspond closely with those by hæmatocrit; $90 \times \frac{52}{40} = 117$, as compared with 118 found.

It will be noted that the blood volume determined by vital red is considerably in excess of the 48 c.c. per kilo. based by some observers on exsanguination experiments. In the above case it is about 63 c.c. per kilo.

(*c*) *Nature and degree of the loss of fluid.* The change in the relative volumes of plasma and corpuscles being thus proved to be wholly due to diminution of the volume of the plasma, it was necessary to enquire what constituents of the plasma were involved in the change. It might be due to abstraction only of water and diffusible constituents, or to actual loss of plasma. The point has an important bearing on the nature of the action. Estimates were therefore made of the composition of the plasma before and during the shock. The proteins being the constituents concerning which information was required, determination of the refractive index was considered a sufficient indication. In one experiment part of each blood sample was oxalated with a constant proportion of solid potassium oxalate and plasma obtained; part was allowed to clot and the serum separated for estimation. In another experiment all samples were taken without oxalate and measurements made on serum only. In neither case was any important change detected in the protein content. The results of the second experiment may be quoted.

*Cat weighing* 3·8 *kilo.*

| | Arterial blood-pressure | Hæmoglobinometer | Protein percentage in serum by refractometer |
|---|---|---|---|
| 3.43 p.m. | 240 mm. | 88 | 8 |
| 3.45 „ | 4 mgm. histamine | | |
| 3.46 „ | 140 | 94 | 7·7 |
| 3.47 „ | 86 | 114 | 7·3 |
| 3.50 „ | 78 | 130 | 7·0 |
| 3.52 „ | 90 | 124 | 7·0 |
| 3.57 „ | 92 | 121 | 7·1 |
| 4.11 „ | 154 | 108 | 7·0 |
| 4.19 „ | 144 | 104 | 7·0 |

It will be seen that the shock-effect in this case was of a relatively weak and very rapidly evanescent nature, but the corpuscular content of the blood nevertheless showed a remarkable increase. At the same time the serum showed no increase of its protein. There was, indeed, a small decrease, the occurrence of which can only be explained by supposing that loss of plasma from one part of the circulation was accompanied by some absorption of water in another part. A similar reduction in the volume of the plasma, accompanied by lessening of the proportion of its solid constituents, has been recorded in traumatic shock. The main point, however, is the absence of any concentration of the plasma. The hæmoglobin values indicate a loss of nearly one-half of the plasma volume, so that if the diminution were due to abstraction of diffusible constituents only, the protein content should be nearly doubled instead of slightly diminished.

In the case quoted, in which the volume of the plasma was directly determined by vital red, it diminished from 87 c.c. to 51 c.c.—a loss of 41 p.c. In other instances an even greater proportionate loss can be calculated from the hæmoglobin or hæmatocrit figures. Thus, in the experiment detailed above, the hæmoglobin value rose from 88 to 130. If we assume a normal corpuscular volume of 33 p.c., this rises to $33 \times \frac{130}{88} = 49$ p.c. Representing the constant volume of corpuscles as 1, the volume of plasma declines from 2 to $\frac{51}{49}$—a loss of practically one-half of the original volume. In another instance the hæmoglobin value rose from 70 to 122, and the corpuscular volume from (say) 33 p.c. to 57 p.c. Again taking the volume of corpuscles as 1, we have the plasma volume declining from 2 to 0·75, *i.e.* a loss of 60 p.c. of the plasma originally present.

(*d*) *Location of the effect.* In earlier papers we have shown that the evanescent vasodilator effect of small doses of histamine is not limited to any particular organ or tissue. It seemed possible, however, that the loss of plasma from the blood would have a less general distribution. From analogy with the action of peptone, the effect of which in producing accelerated flow of lymph in the dog was shown by Starling to be due mainly to action on the liver, it seemed possible that the lymphagogic effect of histamine in the cat would show a similar restriction. We, therefore, in a series of experiments, diverted the blood from the liver by the method which we have recently described (5). When the action of histamine was tested with the liver thus excluded we observed, in one experiment, a fall of blood-pressure of relatively small extent, with practically no change in the proportion of corpuscles, and rapid recovery of the blood-pressure to little below its original level. In subsequent experiments of the same kind, however, we observed the typical, lasting shock-effect after histamine, with pronounced increase in the proportion of corpuscles. We had a similar experience in testing the effect on the action of histamine of removing the whole of the abdominal portion of the alimentary canal and tying off the liver. In one case the eviscerated animal showed a relatively small and evanescent fall of blood-pressure, with a small loss of plasma from circulation, as the result of a large dose of histamine; in other similar experiments, however, a typical shock was produced, with an increase of the corpuscular content of the blood not less than that seen in some of the cases quoted above, in which the abdominal viscera were in normal connexion with the circulation. The following are details from two such experiments, in which about 1 mgm. of histamine base per kilogram was injected after removal of the stomach, intestines and spleen and exclusion of the liver from circulation.

| Arterial pressure | | Hæmoglobin per cent. | |
|---|---|---|---|
| Before | After injection of histamine | Before | After |
| 173 mm. | 40 mm. | 92 | 130 |
| 143 | 70 | 80 | 96 |

We have met with cases of unusual resistance to the effect of histamine also in cats with the abdominal viscera in normal connexion with the circulation; indeed, at one period we encountered several such cases in successive experiments, in which even several milligrams of histamine per kilogram caused only a temporary fall of pressure, without extreme reduction of cardiac output, and with only slight increase in the proportion of corpuscles. We do not know the reason of this variability of the reaction, but its occurrence under normal conditions deprives of significance the occasional failure of the "shock" in the eviscerated animal. The fact that a typical effect is often seen after removal of the bowels shows that the blood vessels in general are liable to the action, and those of the abdominal viscera not conspicuously more so than others.

(*e*) *Effect on the other cellular elements in the blood.* We found that the proportion of leucocytes in the arterial blood underwent a conspicuous diminution during histamine shock, even when that of the red corpuscles was greatly increased; that is to say the effect of the loss of plasma was more than compensated by loss of leucocytes. The diminution was due largely to loss of polymorphonuclear cells, the eosinophiles being reduced to a smaller extent, and the lymphocytes hardly at all. In other words the different types of cells were affected in the order of their amœboid activity. The magnitude of the change in the leucocytic count had no relation to the extent of the loss of plasma; it was at least as conspicuous when the shock was produced by slow infusion, with but little concentration of red corpuscles, as when great concentration occurred as the result of a single large injection of histamine. We do not regard the phenomenon as having any specific significance, since we have observed a leucopenia of the same type as the result of a prolonged fall of blood-pressure produced in other ways—*e.g.* by complete pithing or by slow infusion of acetyl-choline. Presumably any condition of low arterial pressure, with resultant slowing of the blood-flow, causes a tendency for the more amœboid corpuscles to adhere to the endothelium of the vessels and to pass, for the time being, out of the current of circulating blood. It may be recalled that a similar leucopenia was described by Biedl and Kraus(8) in anaphylactic shock in the dog, and has long been familiar in the very similar condition produced in the same animal by peptone. It was stated, however, that in these cases the polymorphonuclear leucocytes accumulated especially in the capillaries of the liver, the organ principally affected by poisoning of these types in the dog.

Another phenomenon, the significance of which is difficult to appraise, deserves mention, since we have repeatedly observed it in blood from animals in histamine shock. If a film is made from such blood and stained in the ordinary way, it is seen to contain numerous large clumps of agglutinated platelets. We received a decided impression that the platelets were not merely agglutinated but abnormally abundant, even in proportion to the increased content of red corpuscles, but we have no quantitative data. In 1914 v. Behring(9) published a preliminary note on some incomplete observations made in his laboratory, according to which an agglutination of platelets occurs in anaphylactic shock. He was inclined to attribute a central importance to the phenomenon, ascribing the symptoms of the anaphylactic shock to cerebral capillary embolisms produced by these clumps of platelets.

4. *Relation of the blood changes to the shock.* The changes in the corpuscular content of the blood are sufficiently striking to raise the question whether they afford an adequate explanation of the phenomena of the shock, or of any part of these phenomena. We have seen that the rapid increase in the proportion of red corpuscles is entirely accounted for by the loss of plasma from the blood vessels. This loss, as calculated from the change in hæmoglobin value or relative corpuscular volume, is frequently of such dimensions that the mere reduction of the volume of

the blood must have a serious effect on the efficiency of the circulation. For example, in one experiment quoted the relative corpuscular volume rose from 30 to 50 p.c. Assuming that the volume of the corpuscles remains stationary, this means a loss of four-sevenths of the plasma. The cat weighed 2·6 kilo., and may be taken to have had about 160 c.c. of blood. Of this 70 p.c. or 112 c.c. would originally be plasma, and 48 c.c. corpuscles. During the shock the volume of the plasma became equal to that of the corpuscles, *i.e.* 48 c.c., so that 64 c.c. had left the circulation. The volume of the circulating fluid, in other words, underwent a reduction of 40 p.c. Now such a reduction of volume, produced by simple hæmorrhage, causes a fall of the blood-pressure to a low level; according to Bayliss spontaneous recovery of the blood-pressure to its initial level does not occur in a decerebrated or anæsthetised cat after bleeding to this extent. On the other hand, the type of circulatory depression produced by such a hæmorrhage is not that produced by histamine; there is not in the former case the extreme reduction of cardiac output and accumulation of the remaining blood at the periphery which histamine produces. It must be remembered, however, that after hæmorrhage in the normal animal fluid is abstracted from the tissues and passes into the blood, so that the volume of the circulating fluid is at least partially restored, and its viscosity diminished. In histamine shock such a restorative mechanism cannot come into play; the loss of volume is due to the opposite process of passage of fluid from the blood into the tissues.

It might well be urged, moreover, that in histamine shock the effect of reduced volume is complicated by that of increased viscosity of the blood, caused by the rise in the proportion of red corpuscles and possibly by obstruction to flow through the capillaries by aggregation of platelets. Thickening of the blood by increase of corpuscular content, and increase of peripheral resistance by partial blockage of capillaries, would not by themselves cause collapse of the arterial pressure. The increase of the peripheral resistance from both causes would, on the contrary, cause the arterial pressure to rise if the output from the heart were maintained. It could not, however, be assumed that it would produce the same effect when associated with a serious reduction of the blood volume. It might be argued that the lowering of the cardiac output would so reduce the arterial pressure that it would be inadequate to drive the thickened blood through the peripheral resistance, so that blood would tend to accumulate in the capillaries, the return of blood to the heart would become deficient, and the output would further decline.

It is very probable that a vicious circle of this type does, indeed, play some part in the production of histamine shock, as seen in many of our experiments. It is easy, however, to show that this combination of lowered volume and increased viscosity is not the only factor in the production of the condition, or even the most important one. A consideration of the figures in Table II will make it clear that there is no close correspondence between the degree of concentration and the severity of the circulatory depression. The lack of correspondence, however, is even greater than the figures in this table suggest. It is possible to produce a condition of severe shock by injection of histamine, without any material change in the relative volumes of red corpuscles and plasma in the general circulation; on the other hand, cases have occurred in our experience, though rarely, in which, while the blood changes were pronounced, the animal escaped such impairment of the circulation as could be held to constitute "shock."

We may consider first the occurrence of shock without significant concentration. Cases of this kind have occurred even when the histamine was injected rapidly, in a single large dose, but these have been exceptional. On the other hand when histamine is given by slow infusion, so that its entry into the circulation is spread over 20 to 30 minutes, absence of serious concentration is the rule, though the shock may be pronounced. One experiment illustrating this point has already been quoted, but it will be of interest to give here the details of another, in which measurements of blood volume were carried out. Such measurements bring to light the very significant fact that, even when the corpuscular content gives no evidence of loss of plasma in the histamine shock, the volume of blood in effective circulation is nevertheless reduced.

**Cat weighing 2·5 kilo. Ether anæsthesia. Record of carotid blood-pressure. Blood samples from the other carotid.**

**3.53 p.m.** B.P. 224 mm.—took 6 c.c. of blood. Hæmoglobin 88 p.c. Hæmatocrit 38 p.c.
**3.58 „** Inject 1 c.c. vital red (1 p.c.) by femoral vein.
**4.1 „** B.P. 156 mm. Bled 6 c.c. for colorimetric determination. Hæmatocrit 37·5 p.c. Plasma volume (by colorimeter) 100 c.c. Blood volume 160 c.c. Animal left (under ether) till 4.55 p.m.
**4.55 „** B.P. 144 mm. (steady). Hæmoglobin 80 p.c. Hæmatocrit 35 p.c.
**4.57 „** Began to run in 0·05 p.c. histamine solution very slowly. Blood-pressure fell rapidly.
**5.4½ „** 2 c.c. (=1 mgm.) infused. B.P. 62 mm. Respiration fails. Artificial respiration on.
**5.14 „** 4 c.c. (=2 mgm.) infused. B.P. 64 mm.
**5.26 „** 8 c.c. (=4 mgm.) infused. B.P. 62 mm. Output of heart very small. Stop infusion. Irregular convulsive inspirations.

5.27 p.m. Took 6 c.c. of blood. B.P. falls to 45 mm. Output hardly perceptible on manometer. Hæmoglobin 90 p.c. Hæmatocrit 39·5 p.c.
5.28 „ 1 c.c. of vital red (1 p.c.) by femoral vein.
5.31 „ Bled 6 c.c. for colorimeter. B.P. falls to 32 mm. Irregular inspiratory gasps continue during artificial respiration. Output of heart almost imperceptible.

Plasma volume (colorimeter) = 73 c.c. Hæmoglobin = 90 p.c. Blood volume = 120 c.c.

There are several points in this record deserving careful attention. The shock produced by the method of slow infusion is less severe than that which usually follows a similar dose given rapidly. The addition of a small hæmorrhage, however, intensifies the effect, so that the circulatory failure is ultimately as complete as that produced by sudden injection. Clearly the animal is suffering from defective volume of circulation. This, however, is not at all suggested by the readings of the hæmatocrit or hæmoglobinometer. The final concentration is represented by a hæmatocrit reading of 39·5 p.c. only, as compared with 38 p.c. originally and 35 p.c. after bleeding to the extent of 12 c.c. What proportion of the loss indicated by the colorimeter is due to the loss of plasma which such a small change indicates? The calculation, which is somewhat complicated by the bleedings, may be most simply made as follows.

The reading at 4.55 p.m. shows that the volume lost by the bleeding made for the colorimeter at 4.1 has been made good by the normal dilution of the plasma. The volumes at 4.1 were blood 160 c.c., plasma 100 c.c., corpuscles 60 c.c. 6 c.c. were taken of which $6 \times \frac{6}{16}$ c.c. = 2·25 c.c. were corpuscles. At 4.55, then, we have 57·75 c.c. of corpuscles and 102·25 c.c. of plasma, giving still 160 c.c. of blood, and a hæmatocrit reading of 35 p.c. This rises after histamine to 39·5 p.c., so that the calculated plasma has fallen to $57\cdot75 \times \frac{60\cdot5}{39\cdot5} = 88\cdot5$ c.c., and the blood volume to $88\cdot5 + 57\cdot75$ c.c. = 146·25 c.c. We remove 6 c.c. of blood at 5.27, before the sample taken for the colorimeter, without further disturbance of the proportion of corpuscles, so that this volume may be simply subtracted, giving us, at the time of the bleeding for the colorimeter, a volume of just over 140 c.c., as estimated from the loss by concentration and bleeding. The determination by vital red, however, shows a volume of only 120 c.c.—*i.e.* it indicates a loss in effective blood volume of 40 c.c., of which only 20 c.c., just one-half, are accounted for by bleeding and by the loss of plasma indicated by the concentration.

It is clear that such a discrepancy must mean either that the vital red does not determine the whole blood volume in these circumstances, or that the hæmatocrit does not give a true indication of the average corpuscular content of the blood in the body. Possibly both sources of error are present. Both would ultimately depend on the stagnation of part of the blood at the periphery. The vital red method determines the volume, the hæmatocrit the corpuscular content, only of the blood which is in effective circulation.

The converse condition, of pronounced concentration of the blood associated with but slight symptoms of shock, has occurred also in our series of experiments. In the following experiment a striking and rapid increase of corpuscular content was associated with only a slight depression of the circulation.

Cat weighing 2·1 kilo. Chloroform, then ether. Cannulæ for record and injection as usual. Vagi cut.

5 p.m. B.P. 160 mm. Blood sample 1 for estimation. Hb. = 74 p.c.

5.6 „ 3 mgm. of histamine intravenously. Small preliminary fall of B.P., then prolonged recovery to about 165 mm. with subsequent slow decline to 120 mm. Manometer pulse reduced, but still such as to show good output.

5.11 „ 4 mgm. of histamine intravenously. Blood-pressure rises briefly to 130 mm. and then sinks slowly to 100 mm. Respiration fails. Artificial respiration applied. Output now obviously reduced, but not very small.

5.17 „ Blood-pressure begins to rise slowly and attempts at natural respiration appear.

5.18 „ Blood sample 2 for estimation. Hb. = 106 p.c.

5.20 „ Spontaneous respiration. Abdomen opened. Bowels congested and somewhat cyanosed. Network of venules clearly visible. Small veins show beaded constriction. Arterioles reduced to small threads. Pancreas dusky in colour, with gelatinous œdema.

The hæmoglobin values indicated the passage of much plasma from the vessels; if the original corpuscular content was 33 p.c. the change represents a loss of about 40 p.c. of the plasma. There was a distinct, though not very pronounced, leucopenia of the usual type, and the customary presence of clumps of platelets in films taken during the action of histamine. The changes in the cellular constituents of the blood and the calculated loss of plasma were, therefore, as great as those seen in most cases of severe shock, and greater than in many. Nevertheless the arterial pressure never fell below 100 mm., the output from the heart, though reduced, never fell to a shock-like minuteness, and both blood-pressure and heart output soon began to recover from even this small degree of depression.

It seems safe to draw the conclusion that the leakage of plasma from the vessels into the tissues, with the reduction in the volume and increase in the viscosity of the blood which it entails, cannot be the main cause of the shock, though it doubtless accentuates its severity. The characteristic features of the condition are due not so much to the fact that the volume of the blood is reduced, as to the tendency of what remains to stagnate at the periphery in the capillaries and venules instead of returning to the heart.

5. *The cause of the peripheral accumulation of the blood.* When we had arrived at the conclusion just stated, it appeared to us that the only type of effect, which could account for the phenomenon we were attempting to analyse, was a relaxation of the tone of the capillary blood vessels. No direct evidence was then available, which would warrant the assumption that histamine could produce an effect of this kind. It has since been provided, however, by the results, already published, of an investigation in which one of us cooperated with A. N. Richards(3). These experiments led to the conclusion that the paradoxical vasodilator action of small doses of histamine, which our earlier work had left unexplained, is due to a direct depression of the normal tone of the capillaries, the plain muscle of the arterioles being always stimulated to increased tone by doses of histamine large enough to affect it at all. Evidence was obtained, in the same series of experiments, in support of the view that other vasodilator substances, of which acetyl-choline was a type, produce their effects entirely on the muscular coats of the arterioles, though the records obtained of their action, with manometer and plethysmograph, showed a remarkable superficial resemblance to similar records of the action of small doses of histamine. According to our view, however, of the nature of the histamine shock, an intensification and prolongation of the action of an arterial dilator, such as acetyl-choline, should produce an effect of an entirely different kind; in particular the accumulation of blood at the periphery, characteristic of the action of large doses of histamine, should not be produced by a vasodilatation limited to the arteries.

We chose acetyl-choline for these experiments, rather than the nitrites, since the latter group cannot be used for producing the prolonged and intense effect which we required, without introducing complications due to their action on the hæmoglobin of the corpuscles. With acetyl-choline a large single dose is unsuitable. As has been shown by Hunt(10) and by Dale(11), with any but minute doses of this substance an intense vagus-like inhibition of the heart is superimposed on the vasodilator effect. Its action, however, is so evanescent, owing to its rapid hydrolysis in the body, that we found it possible, by a carefully adjusted very slow infusion of a dilute solution (0·01 p.c.) into the vein of a cat under ether, to produce a persistent low blood-pressure for upwards of 30 minutes by vasodilatation alone, without causing inhibition of the heart. We have performed the experiment a number of times, with uniform result. The arterial pressure fell rapidly with the commencement of the infusion, and could be kept steady at a level of 40–50 mm. by controlling the rate of inflow, which usually had to be very slightly accelerated as the experiment proceeded. The heart beat rapidly, and usually showed some irregularity of rhythm, but the output remained thoroughly efficient, as judged by the excursions of the manometer and by the general state of the circulation. The intestines showed a pink flush and pulsated obviously, and the great veins were well filled, but not over-distended. Samples of blood taken before the infusion and during its progress showed that a slow dilution of the plasma was taking place. Spontaneous respiration sometimes continued throughout the experiment, but periods of artificial respiration were necessary in some cases. Perhaps the most striking contrast with the effect on the circulation produced by histamine, when similarly administered, was presented by the events following the stoppage of the infusion. After acetyl-choline the arterial pressure began immediately to rise from the low level at which it had been maintained, and with an extraordinary rapidity regained or surpassed the level at which it had stood before the infusion began. After similar treatment with histamine the typical picture is the shock which we have described in detail above. Usually there is in this case no sign of spontaneous recovery; the arterial pressure can be driven up and the output of the heart temporarily restored by compression of the limbs and abdominal vessels, only to fall away with relaxation of the compression, as the blood slowly accumulates again at the periphery, and the heart and great vessels are again depleted.

In favour of our view that the shock-like failure of the circulation is due to accumulation of the blood in relaxed capillaries we have, then, the positive evidence that histamine has been shown to relax capillaries, and the negative evidence that acetyl-choline, which appears to dilate the arterioles only, does not produce a condition in any way resembling shock.

There is one point, however, which needs further explanation. The effect of small doses of histamine, which Dale and Richards attributed to relaxation of capillary tone, presents the typical features of a vaso-dilator action; during the fall of arterial pressure caused by such small doses the output of the heart is increased, as we showed in our first paper on the action (1). The action, in other words, is due to weakening of the peripheral resistance, and is not a volume effect diminishing the return to the heart. In effect, therefore, though not in location, the action of these small doses is practically identical with that of the arterial dilators. Why should the two types of action have outcomes so widely different when both are intensified and rendered persistent?

We believe that the answer to this question is to be found by considering the normal state of the flow of blood through the capillaries. All who have studied it under the microscope have been struck by the comparatively small part of the available capillary network used for conveying the blood under normal conditions. Langley (12) has pointed out how under conditions of low pressure the path of the blood may be changed by the closing down of the capillaries in one area and the opening up of those in another. It may be supposed that, though distribution is constantly changing, the total capillary path available at any moment normally varies but little. A small dose of histamine, the effect of which will spread hardly beyond those capillaries which provide a path for the blood during the brief period of its action, will produce simply an evanescent widening of that path, of which the effect will differ but little from that of an evanescent arterial relaxation. When the effect is intensified or rendered more persistent by large dosage, we suppose that a different set of factors comes into play. Not only the tone of the capillaries in actual use when the effect begins, but that of the whole network potentially available now comes under the action of the drug. With a simultaneous loss of tone of the capillaries throughout the body their capacity for holding blood rises far beyond the normal average, and the blood spreads and percolates into the whole network of patent channels as into a sponge, so that the outflow into the veins and back to the heart at once begins to fail. Constriction of the arterioles will mitigate the increase in the total capacity of the system caused by relaxation of the capillaries, and by maintaining a high peripheral resistance will hold up the arterial pressure, which will only fall gradually as the output from the heart decreases. But though arterial constriction delays the fall of pressure in the arteries, it cuts off that pressure the more effectively from the blood in the capillaries, and with the wane of the output from the heart the flow through the capillaries becomes ever slower and the tendency to stagnation greater, till ultimately only a relatively small part of the available blood is effectively circulating. A relaxation of arterial tone at this stage, whether from the passing of the vaso-constrictor effect of histamine or failure of the anæmic vasomotor centre, will further increase the capacity of a system, for the filling of which the blood is already inadequate.

The true significance of the loss of blood volume owing to escape of plasma can now be appreciated. It may be noted, in the first place, that it is probably another aspect of the same poisonous action of histamine on capillary endothelium. We may suppose that an intensification of the action which relaxes the normal capillary tone renders the endothelium abnormally permeable, so that it no longer retains the plasma. It is readily intelligible, therefore, that the loss of plasma from the blood should be a more conspicuous feature of the effect as produced by sudden introduction of a large dose of histamine than of that produced by slow infusion. It will be observed also that this association of increased vascular capacity and low blood-pressure, with diminution of volume and concentration of the blood, involves the abrogation of one of the normal compensatory processes of the body. Low blood-pressure, as produced by relaxation of arterial tone, is accompanied by a passage of water from the tissues into the blood, reducing the disparity between the volume of the blood and the capacity of the system. If the corpuscular content is estimated in small samples of blood, taken during the fall and recovery of arterial pressure which follow a small dose of amyl nitrite, for example, it is found that the curve of dilution and reconcentration follows that of the arterial pressure with great accuracy. With the fall produced by histamine this compensatory process of dilution is not merely absent, but reversed; the effect of increased capacity is accentuated by decline of blood volume.

While we are convinced that general dilatation of the capillaries and loss of plasma from the blood are main factors in the production of the shock, we are not in a position to exclude others. The stagnation of blood in the capillaries which occurs in inflammation has been thought by some observers to be the result of a change in the endothelium of such a nature as to hinder the flow of blood through the small channel, though no clear conception has been put forward as to what the nature of such a change could be. There are theoretical difficulties in the way of supposing that a mere change in the physical properties of the surface of the endothelial cells could produce such an effect, and Trevan(13) has recently maintained that the stasis is sufficiently explained by local concentration of the blood and consequent increase in its viscosity. We have been unable to put these possibilities to the test of experiment, so that it is sufficient to admit that, if other changes than those which are clearly recognisable play a part in the stasis of inflammation, they are possibly concerned also in the peripheral stasis produced by histamine. It must be recognised that there is no general agreement as to the factors determining the rate of flow through the capillaries. The conditions which we have called "relaxation" and "tone" of capillary walls do not necessarily correspond with the phenomena so called in the case of plain muscle. They may simply represent the effects of imbibition and loss of water by the endothelial cells. Leonard Hill(14) maintains that "the influence on the capillary circulation of osmotic and surface energy can be no less than that effected by the heart and vasomotor system," and points out that "in the active conditions of life the contents of the capillaries are continually being emptied onwards by the contractions of the skeletal muscles, pressure against external bodies and the influence of gravity in changes of posture." As regards surface energy the suggestion is not yet sufficiently definite to enable us to consider whether changes of this kind play any part in the action of histamine; but it can hardly be doubted that the loss of tone and movement of the voluntary muscles will accentuate the tendency to peripheral stagnation when the shock is fully developed. Occasionally an animal in this condition performs a movement of the nature of a convulsive inspiration or a vomiting effort, and the accelerated return of blood to the heart is at once obvious in a temporary rise of arterial pressure and increase of output.

We may summarise our conclusions, as to the cause of the peripheral accumulation of blood after a large dose of histamine, by saying that we regard it as principally the effect of a general relaxation of the capillary vessels; that the loss of blood volume owing to morbid permeability of the endothelium accentuates the effect of this relaxation in producing a relative deficiency of blood volume in proportion to the capacity of the system; that the lack of the normal favouring influence on capillary flow exerted by movement of the voluntary muscles probably plays some part; and that, in addition to the widening of the capillary channels and the thickening of the blood by loss of plasma, there may be other factors favouring stasis in the capillaries which only further investigation can define.

## SOME PRACTICAL APPLICATIONS AND ANALOGIES.

It will hardly have escaped notice that the conception of the action of histamine, to which our analysis has led us, embodies the main features of a reaction which, if it occurred locally, would be recognised as a mild inflammation. The widening of capillary lumina, the opening up of channels normally empty, the transudation of plasma through abnormally permeable endothelium and the tendency to stasis of the concentrated blood—these phenomena, when occurring in an area so restricted that the general arterial pressure is not materially affected, will produce the familiar sequence of redness, swelling and œdema. This, indeed, is the effect of histamine when locally applied, as Sollmann and Pilcher(23) have shown; applied even in high dilutions to the lightly scarified human skin histamine causes redness, swelling and the formation of an œdematous wheal.

In its general action on the other hand, when introduced into the circulation in large doses, histamine reproduces in a very suggestive manner the complex which in recent years has come to be recognised as characteristic of one kind of traumatic shock. The collapse of arterial pressure in spite of arterial constriction, the weakening of the pulse out of proportion to the fall of arterial pressure, the rise in corpuscular content of the blood owing to loss of plasma, the fall in cardiac output, in spite of but slight impairment of the activity of the heart, owing to a fall in effective blood volume in excess of that due to loss of plasma—all these phenomena, as well as the associated depression of the respiratory centre, fall of body temperature and general lethargy, can be found in the descriptions of surgical or traumatic shock by Malcolm(15), Y. Henderson(16), Mann(17) and others. The concentration of the blood by loss of plasma had been earlier recognised by Sherrington and Copeman(24) as a characteristic feature of shock. Some years ago we drew attention to the many points of similarity between the action of histamine and the so-called "anaphylactic shock." The resemblance to some cases of shock following trauma seems to be even closer than to the anaphylactic reaction, which has been called "shock" by analogy.

The parallel becomes even more suggestive when it is remembered that local trauma, when not so severe that its effect is complicated and obscured by effusion of blood from ruptured vessels—for example, a blow on the skin from a cane or a whip—will cause the same sequence of capillary flushing and wheal-formation as the dermal application of histamine. So that we have locally applied histamine reproducing the effect of a mild local injury of the tissues, and histamine distributed through the system reproducing the sequel to a massive injury of tissues.

The action of histamine does not stand by itself, but represents in its most characteristic features a type of action common to a large group of poisonous substances of animal or bacterial origin. Substances having this type of action have been extracted from most of the organs of the body, though it is unlikely that histamine itself is among them, except in special cases such as that of the intestinal mucosa. (Barger and Dale(18).) It may be noted, however, that other substances of this class —*e.g.* bee poison or certain bacterial products—can produce either local inflammation and œdema or profound circulatory collapse, according to the method of application and the magnitude of dosage. A poisoning of the capillary endothelium seems to be the common factor in the action of all. This again brings them into relation with certain metallic poisons, such as arsenic and gold salts, which were long ago classed by Heubner(19) as "capillary poisons," and which, when introduced in appropriate doses into the general circulation, produce a shock-like prostration. The shock-like symptoms which have occasionally followed the intravenous administration of salvarsan probably depend on an effect of this type—an acute endothelial intoxication.

The existence of these points of community, in the action of substances so utterly unrelated chemically as histamine and certain metallic ions, forbids any assumption that the production of similar effects, by unknown constituents of some organ or tissue, indicates the presence therein of histamine itself, or of any substance chemically related to it. The similarity depends on the fact that all act on the endothelium, and produce in it changes probably of the same general type. A hint, as to what the nature of these changes may be, is possibly provided by the fact that the anaphylactic reaction, more especially in those species in which histamine exhibits this type of action, also presents the picture of an acute endothelial poisoning. All available evidence goes to show that the anaphylactic antibody is of the nature of a "precipitin," the interaction of which with the corresponding antigen results in a change in the state of dispersion of the colloidal particles. One of us (Dale(25)) has given reason for believing that the occurrence of this reaction actually in the plain muscle fibres of the guinea-pig is the exciting cause of the contraction of the plain muscle, which gives to the anaphylactic "shock" its characteristic type in that species. It is not inconceivable that a physical change of this type occurring in the endothelial cells would lead to relaxation of their tone and ultimately to abnormal permeability; indeed if the anaphylactic reaction, as exhibited in different tissues and species, depends always on the same type of interaction between antigen and antibody, this would almost of necessity follow. So that it is quite possible that the production by histamine, and by a whole group of other substances, of a complex including contraction of plain muscle with relaxation and permeability of capillaries, may depend on a common type of physical change in protoplasm produced by all of them, the result of which receives different expression in terms of the physiology of different tissues. At this point, however, speculation without further basis of fact becomes fruitless.

A new and practical importance has been given to this type of action by recent work of Bayliss(20) and of Cannon(21). We have pointed out above how the local effects of injury may resemble those of histamine. Bayliss and Cannon have provided a rational basis for the similarity, mentioned above, between the shock produced by histamine and that following trauma, by direct evidence that shock follows extensive injury to the tissues of a limb, the nervous connexion of which with the rest of the body has been severed, but that it is prevented by clamping the blood vessels. They conclude that an important role in the genesis of shock is played by absorption into the general circulation of products liberated by the injury of tissues—their experiments deal especially with skeletal muscle—having the type of action which we have been discussing. Delbet and Quénu(22) have independently developed similar views of the importance of a chemical factor in shock. The presence of large quantities of substances having this action in the contents of the stomach and intestine is suggestive in connexion with the pronounced association of shock with injuries of those viscera, and on the ease with which rough handling or excessive exposure of them determines its onset. Whatever may be the ultimate conclusion as to the importance of such factors in surgical shock, the recognition of this traumatic toxæmia provides an important addition to the points of community in causation, which are gradually coming to light, between a number of physiological and pathological phenomena, the symptomatic similarity of which has long been recognised.

## SUMMARY.

In doses of the order of 1 mg. per kilogram and upwards histamine produces in the anæsthetised cat a condition presenting numerous points of resemblance to traumatic and surgical shock. The central feature of the condition is an oligæmia, partly due to passage of plasma out of the blood vessels, partly to retardation of the flow of blood at the periphery. Evidence is given in favour of the view that this is due to a general loss of tone by the capillaries throughout the body. The excessive permeability of these vessels, which allows escape of plasma, is regarded as a further stage of the same type of action. This type of effect appears in the action of a large number of substances, some of them having no kind of chemical relationship with histamine. The nature of the common factor in the effect of these, and its relation to the similar symptoms seen in the anaphylactic reaction, in shock, etc., are discussed.

We are greatly indebted to Miss F. M. Durham and to Major C. H. Kellaway, M.C., A.A.M.C. for assistance in some of the experiments. Also, in a special degree, to Prof. A. N. Richards, of the University of Pennsylvania, who was prevented by the call of a more urgent duty from extending, as we had hoped, to the completion and publication of this work the invaluable cooperation which he gave at one stage.

## REFERENCES.

(1) Dale and Laidlaw. This Journal, **41**. p. 318. 1910.
(2) Dale and Laidlaw. Ibid. **43**. p. 182. 1911.
(3) Dale and Richards. Ibid. **52**. p. 110. 1918.
(4) E. Mellanby. Quart. Journ. of Med. **9**. p. 165. 1916.
(5) Dale and Laidlaw. This Journal, **52**. p. 351. 1919.
(6) Fühner and Starling. Ibid. **47**. p. 286. 1913.
(7) Keith, Rowntree and Geraghty. Arch. of Int. Med. **16**. p. 547. 1915.
(8) Biedl and Kraus. Wiener Klin. Woch. **22**. p. 363. 1909.
(9) v. Behring. Deutsch. med. Woch. **40**. p. 1857. 1914.
(10) Hunt. Amer. Journ. Physiol. **45**. p. 197. 1918.
(11) Dale. Journ. Pharm. and Exp. Ther. **6**. p. 147. 1914.
(12) Langley. This Journal, **41**. p. 483. 1911.
(13) Trevan. Biochem. Journ. **12**. p. 60. 1918.
(14) Hill. Further Advances in Physiology, pp. 134–135. London, 1909.
(15) Malcolm. Lancet, 1905, ii. p. 573. Also in "The Physiological Basis of Traumatic Fever." London, 1893.
(16) Y. Henderson. Amer. Journ. Physiol. **27**. p. 152. 1910.
(17) Mann. Surg. Gyn. and Obst. **21**. p. 430. 1915. Amer. Journ. Physiol. **47**. p. 231. 1918.
(18) Barger and Dale. This Journal, **41**. p. 499. 1911.
(19) Heubner. Arch. f. exp. Path. u. Pharmakol. **56**. p. 370. 1907.
(20) Bayliss. Oliver Sharpey Lectures. 1918.
(21) Cannon. C. R. Soc. de Biol. **81**. p. 850. 1918.
(22) Quénu, also Delbet. Ibid. **81**. p. 858. 1918.
(23) Sollmann and Pilcher. Journ. Pharm. and Exp. Ther. **9**. p. 309. 1917.
(24) Sherrington and Copeman. This Journal, **14**. p. 83. 1893.
(25) Dale. Journ. Pharm. and Exp. Ther. **4**. p. 167. 1913.

# THE RATE OF DIFFUSION OF GASES THROUGH ANIMAL TISSUES, WITH SOME REMARKS ON THE COEFFICIENT OF INVASION. BY AUGUST KROGH.

(*From the Laboratory of Zoophysiology of Copenhagen University.*)

THE rate at which gases and especially oxygen will diffuse through tissues has, so far as I am aware, never been systematically investigated and practically never been investigated at all, though a knowledge of the gas diffusion is obviously essential for the solution of one of the problems of the physiology of to-day: the supply of oxygen to the cells. The brilliant work of Barcroft and his collaborators has made it comparatively easy to obtain a quantitative idea of the average oxygen tension in the capillaries and has furnished many of the necessary data concerning the call for oxygen of the tissues, but in order to make out how this call can be met it is necessary (1) to measure and calculate the average distances which the oxygen molecules have to travel from the capillaries until they enter into chemical combination, and (2) to know the rates at which they travel, that is the diffusion coefficients for oxygen in the different tissues. The present paper is intended to supply the second of these desiderata for certain tissues.

The diffusion of gases through animal tissues must take place in essentially the same way as their diffusion through fluids or colloidal membranes. The gases are dissolved in the tissue fluids and diffuse in a liquid state. The laws governing the diffusion of gases through water and watery solutions have been worked out by Exner(1), who found that the rates of diffusion for different gases in the same fluid are proportional to the absorption coefficients of the gases in the fluid and inversely proportional to the square roots of their molecular weights. Exner could only measure relative diffusion rates for different gases. Stefan(2) measured directly the rate of diffusion of carbon dioxide. Hüfner(3) devised a method for measuring directly the diffusion rates of different gases through water. He defined the diffusion coefficient of a gas as the quantity diffusing through 1 square cm. and 1 cm. thickness in 24 hours, when the pressure difference is 1 atmosphere, divided by the absorption coefficient for the gas in question. This unit is very unpractical for physiological work, the more so as the absorption coefficients for gases in tissues are generally unknown and their accurate determination very difficult. I prefer therefore to define the diffusion constant simply as the quantity diffusing through 1 sq. cm. and $1\mu$ (0·001 mm.) thickness in 1 minute at a pressure difference of 1 atmosphere. For comparison purposes Hüfner's measurements of diffusing quantities must be multiplied by $\frac{10000}{1440}$ and his coefficients further by the absorption coefficients of the gases in water. Hüfner found for oxygen in water a diffusion coefficient of 1·62 or, expressed in my terms, a diffusion constant of 0·34. Later Hagenbach (4) measured the diffusion of gases through gelatine of about 20 p.c. concentration. He found that most gases diffuse somewhat more slowly through gelatine than through pure water, the diffusion coefficients being on an average only 67 p.c. of those found by Hüfner for water. For oxygen he obtained a value which was very much higher than anticipated namely 1·6 expressed in my terms instead of 0·23. As animal tissues must *a priori* be assumed to show properties similar to those of gelatine a renewed investigation is obviously necessary.

*Methods.* In my researches on the diffusion rates of gases through tissues I have employed two fundamentally different methods. The first involves the diffusion of a gas ($O_2$, CO or $CO_2$) either from one fluid through a tissue membrane to another fluid or from air through the membrane to a fluid. The second involves simply the diffusion of two pure gases from either side of a tissue membrane to the other. The use of the first method appeared necessary in order to settle certain questions about invasion which will have to be dealt with in their place. The second method is by far the simpler and easier of the two and, as it turned out, the one giving the most reliable results. I shall describe the methods and the results obtained by each of them separately.

1. The apparatus[1] which is made entirely of brass and gilded inside consists of two vessels *A* and *B* which can be put firmly together by means of three binding screws. *A* has a capacity of 1·5 c.c. It can be filled through the tubes 1 and 2 with the fluid into which the diffusion is to take place. *A* is separated from *B* by the diffusion membrane which

---

[1] I am indebted for several important details in this apparatus to the constructive skill of the laboratory mechanician, Mr H. Pedersen.

is prepared as follows. A piece of suitable membranous tissue is stretched out on a cork plate over a brass ring 3 which is of such a size that it fits loosely on the outside of the slightly projecting circular orifice of *A*. A second ring 4 which is slit open on one side is pushed down on 3 and secures the piece of membrane to be employed. When *A* and *B* are screwed together a circular area of the membrane becomes available for diffusion. The size of this area is in my apparatus 0·794 sq. cm. *B* has been made in two pieces which are screwed apart for cleaning purposes. It will hold about 50 c.c. A gas can be led in through the tube 5 and when a fluid is employed in *B* a froth chamber is usually placed on the tube 6. In both vessels adequate mixing arrangements are provided and special precautions have been taken to secure the completest possible renewal of the fluid along both surfaces of the membrane. The mixing screws are driven by a small motor at the rate of 1000 to 2000 revolutions per minute. During experiments the apparatus is placed in a large air thermostat and a constant temperature maintained.

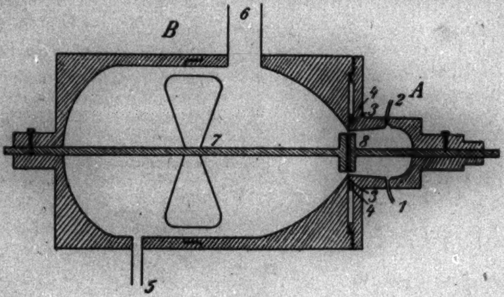


Fig. 1.

Diffusion experiments have been made chiefly with oxygen and a constant oxygen pressure of 1 atmosphere was maintained in *B* by allowing a current of the gas (98·0 p.c.) to pass through at a uniform but generally slow rate. *B* was in some experiments filled with 40 c.c. blood (or hæmoglobin solution) while in others it contained only gas, saturated with moisture. *A* was at the beginning of each experiment filled with reduced blood (or hæmoglobin solution). The reduction was easily carried out by bubbling pure nitrogen through the solution in the vacuum obtained by a good filter pump. During the filling of *A* two 0·5 c.c. samples of the reduced solution were taken and the amount of gas which they would take up from air at a temperature of 17° was determined by means of Barcroft's method[1].

During the experiment oxygen will diffuse from *B* to *A* through the membrane. In *A* the oxygen will enter into combination with the hæmoglobin. At the low temperature employed the tension of dissociation of the oxyhæmoglobin formed is so low that it can be left out of account, at least so long as the hgbl. is not more than half saturated. We have on one side therefore a constant $O_2$ pressure of one atmosphere—due allowance being made of course for the vapour tension and the small impurity of the oxygen—while the pressure on the other side remains at 0. After a suitable time two 0·5 c.c. samples are taken from *A* and the amounts of oxygen which they will take up from air determined as before. The differences between these determinations and those made at the beginning of the experiment give, when multiplied by the volume of *A*, the quantity of oxygen which has traversed the membrane during the experiment.

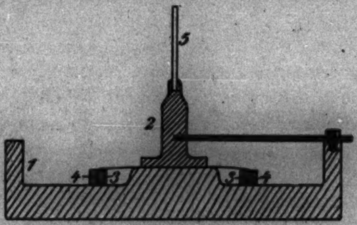


Fig. 2.

In order to obtain the rate of diffusion per unit length of way it was necessary further to measure the thickness of the membrane. This was in most cases carried out by means of the simple device shown in Fig. 2. The membrane with the rings 3 and 4 was placed in saline in the small circular trough 1 which was mounted on a horizontal microscope. The small weight 2 was placed upon it. This weight carried a glass plate 5 on which a horizontal and a vertical fine line were engraved. The microscope was provided with a screw micrometer eyepiece. The vertical line on the glass plate was made to coincide with the vertical thread in the micrometer and the position of the horizontal line was measured alternately with the weight standing directly on its small platform in the trough and with the membrane interposed. Usually a series of at least ten alternate readings were taken and in most cases a very good agreement was obtained.

---

[1] A description of certain small and not very important modifications of Barcroft's differential blood gas analysis, which we have introduced in this laboratory, has been given by **Andresen** (5).

Most of the diffusion experiments were made with oxygen but some also with carbon monoxide and a few with carbon dioxide. In the experiments with $CO_2$ a solution of sugar isotonic with normal saline and containing 0·005 p.c. NaOH was used instead of blood, and air with an analysed content of 6—10 p.c. $CO_2$ was employed instead of the pure gas because the diffusion would otherwise become too rapid for accurate or convenient measurement. The carbon dioxide taken up by the alkaline solution was determined in the Barcroft apparatus in the usual way.

As an example to show the experimental procedure and the manner in which the results are calculated I reproduce the protocol of one of the determinations.

Bar. 762·5 Tp. of diffusion apparatus 16·0°.

Membrane: abdominal wall of frog (muscles and connective tissue), thickness 221 $\mu$.

Fluid: corpuscles of ox blood, washed with NaF, dissolved in water. Added 0·9 p.c. NaCl. In *B* hgbl. solution treated with a current of 98·0 p.c. $O_2$. The solution for *A* reduced (incompletely). The reduced solution run into *A* through 1 at 11.17. Two samples of 0·5 c.c. of the solution passing out through 2 put into Barcroft bottles (*a* and *b*). Mixing started, diffusion exp. begun 11.18. Stopped at 3.40. Duration of exp. 262 m. Two samples from *A* through 1 into Barcroft bottles (*a* and *b*). The determinations gave

| | | | | | |
|---|---|---|---|---|---|
| Taken up from air c.mm. | $a$ | 62·9 | $b$ | 60·0 | |
| | $a_1$ | 28·8 | $b_1$ | 27·5 | |
| Difference | | 34·1 | | 32·5 | or for 1 c.c. 66·6. |
| Liberated with FeCy | $a$ | 77·7 | $b$ | 77·5 | |
| | $a_1$ | 75·0 | $b_1$ | 75·9 | |

In the final samples the volumes of $O_2$ liberated with FeCy are on an average 2·1 c.mm. less than in the initial. This must mean that a corresponding amount of methæmoglobin has been formed in *A* during the experiment and as the oxygen for this has probably passed through the membrane the corrected difference per c.c. is 70·8 c.mm. In most experiments this correction is insignificant or absent. The oxygen pressure in *B* has been $\frac{98}{100}(762\cdot5 - 13) = 734$ mm. The volume of $O_2$ which has diffused into *A* is $0\cdot0708 \times 1\cdot47 = 0\cdot104$ c.c. that is per minute and sq. cm. through a thickness of 1 $\mu$ and with a pressure difference of 760 mm. $\frac{0\cdot104}{262} \cdot \frac{221}{0\cdot794} \cdot \frac{760}{734} = 0\cdot114$ which is called the diffusion constant for the membrane in question.

In this case and in a few others with very thick muscular membranes a slight correction has to be applied, because the membrane has used up oxygen during the experiment. The respiratory exchange of such membranes has been determined in special experiments and it has been assumed that the amount of oxygen used has on an average diffused through half the thickness of the membrane. The correction will raise the diffusion constant to 0·117. A second experiment of 234 minutes duration on the same membrane gave also the value 0·117 for the diffusion constant for $O_2$ while an experiment with CO lasting 260 minutes gave 0·082 for this gas.

The membranes investigated by this method were the following:

(*a*) A rubber membrane of $37\mu$ thickness.

(*b*) The muscular wall from the sides of the abdomen of a frog. A very uniform piece of tissue can be obtained consisting of two layers of connective tissue each between 15 and $30\mu$ thick and a much thicker layer of striped muscle.

(*c*) In two experiments the muscular tissue was removed from the abdominal wall by gentle scraping and the diffusion through the connective tissue alone measured. One of these experiments was made with the double layer of connective tissue from a large frog, measuring $63\mu$, the other with a single layer from a small frog measuring only $17{\cdot}5\mu$.

(*d*) Several experiments were made finally with a membrane consisting chiefly of smooth muscle from the "uterus" of a pregnant frog.

(*a*) On rubber three separate determinations were made with oxygen giving for the diffusion constant 0·066, 0·062 and 0·064 respectively, average 0·064; three determinations with CO gave 0·038, 0·034 and 0·036, average 0·036, while a single experiment with $CO_2$ gave 0·32. All the experiments were made at temperatures between 16° and 17°.

A calculation of some of Graham's (6) experiments, in which atmospheric air diffused through rubber into a vacuum and the resulting gas mixture was measured and analysed, gives for oxygen:

| | | |
|---|---|---|
| Rubber tube of 2 mm. thickness (p. 567) Tp. 20°–23° | Diff. constant | 0·050 |
| Sheet rubber of 1 mm. thickness (p. 568), Tp. 20° | „ | 0·059 |
| Rubber balloon said to be 0·02 mm. thick* (p. 569) | „ | 0·062 |

* The weight and diameter of this balloon have been given. Assuming a specific gravity of 0·92 the thickness works out as 0·029 mm. This would increase the diffusion constant to 0·090.

Graham has found the following relation between the diffusion constants of different gases. A comparison with my values shows an agreement which must be considered as satisfactory. My figures have been obtained on rubber saturated with moisture, **Graham**'s on the dry substance:

| | Graham | Krogh |
|---|---|---|
| Nitrogen | 0·39 | — |
| Carbon monoxide | 0·44 | 0·56 |
| Methane | 0·84 | — |
| Oxygen | 1·0 | 1·0 |
| Hydrogen | 2·15 | — |
| Carbon dioxide | 5·3 | 5·0 |

(*b*) The experiments on muscle have been given in detail above.

(*c*) The connective tissue membranes. Two determinations were made on each with oxygen. In one of these the diffusion took place from gas to blood and in the other from blood to blood, but this difference apparently had no influence upon the rate of diffusion, though in the experiments gas-blood an invasion of $O_2$ into the surface of the membrane had to take place in addition to the diffusion. The significance of this fact will be discussed below. The results were:

| | Tp. | Diffusion constant | $O_2$ | CO | $CO_2$ |
|---|---|---|---|---|---|
| Connective tissue 17·5μ thick | 16° | Gas-blood | 0·095 | 0·069 | — |
| | | Blood-blood | 0·087 | — | — |
| Connective tissue 63μ thick | 18° | Gas-blood | 0·106 | — | — |
| | 20° | Blood-blood | 0·118 | 0·083 | 4·0 |

(*d*) On the frog's uterus I found it impossible to obtain constant results, and the diffusion constants found were in all cases much lower than in striped muscle or connective tissue. The reason for this was revealed by the histological examination of the membrane which showed that its surface was very uneven. A reliable determination of its effective thickness was therefore impossible. The following experiments were made:

| | | Diffusion constant | |
|---|---|---|---|
| Thickness | Tp. | $O_2$ | CO |
| 24μ | 15° | 0·079 | 0·043 |
| 21μ | 36° | 0·069 | — |
| 15·5μ | 16° | 0·038 | — |
| | 36° | 0·050 | — |
| | 36° | 0·037 | — |
| | 17° | 0·035 | — |

2. The results obtained on all the tissues examined by the first method are much lower than **Hüfner**'s on water, viz. 0·34 for oxygen, and I suspected therefore that there might be some unknown source of systematic error in my experiments. To investigate this point and also

in order to study the diffusion at different temperatures I worked out the second method briefly mentioned above. Two glass vessels of approximately equal volume (35 c.c.) and the form shown in Fig. 3 are at 1 provided with metal flanges exactly similar to those used for mounting the diffusion membrane in the first series of experiments. The lower end of both vessels is connected with a single mercury reservoir (not shown in the figure). By raising this mercury can be run into both vessels simultaneously and at the same rate. Through the tube 2 each vessel can be washed out with a current of pure gas—oxygen or nitrogen—saturated with moisture. The upper ends of both vessels are connected with the end of glass tubes 3 of 1 mm. bore carrying two three-way taps. By means of these taps the vessels can be arranged to communicate through the tube 4 containing a long drop of kerosene acting as a manometer. The free branches of the three-way taps are utilised for connecting with gas sampling vessels. These are of 20 c.c. capacity and of the type usually employed in this laboratory, but the pair of them are connected below through a wide Y-piece with a single mercury reservoir in order to secure a uniform pressure in both during the taking of samples. 5 are needles of steel enclosed in glass tubes provided with plates of mica. The needles can be moved up and down from outside by means of a magnet, and the mica plates serve for the thorough mixing of the gases in each vessel before the taking of samples.

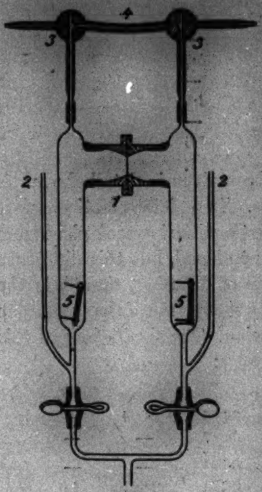


Fig. 3.

An experiment is made as follows. A suitable membrane is mounted in the rings 3 and 4 (Figs. 1 and 2). The thickness is measured and it is put in place between the two vessels (1, Fig. 3). The apparatus is placed in a constant temperature water-bath. A current of pure nitrogen is led through the left-hand vessel and a current of nearly pure oxygen through that to the right. Care is taken that the spaces on each side of the manometric drop are also filled with nitrogen and oxygen respectively. When the vessels are completely washed out with the pure gases—about 1 l. gas has generally been used for this purpose—samples are taken from the current on both sides. The vessels are closed at the bottom by raising the mercury above the junctions, and at the top they are connected with the manometer. The diffusion experiment is taken to begin at the moment when the gas currents are interrupted. The diffusion of oxygen through a membrane being more rapid than that of nitrogen the drop of kerosene will move from left to right, and the rate at which it moves gives some indication of the diffusion rate. The levels of mercury in the two vessels are adjusted from time to time so as to maintain the same pressure throughout in both vessels.

When the experiment has to be finished (several times also during its course) the gases in each vessel are thoroughly mixed. The taps 3 are closed. Mercury is run into both vessels and when a positive pressure has been obtained the taps are opened to wash out the connections (lead tubes of very narrow bore) down to the sampling vessels and a sample of 20 c.c. is drawn from each side of the membrane. The experiment is considered as finished when the first half of the samples has been collected. In most cases the experimentation time has been so long that the unavoidable uncertainties about the time of beginning and finishing the experiment have been unappreciable. The samples drawn are analysed for oxygen and nitrogen respectively. In a few cases with very thick muscular membranes the $CO_2$ liberated by the respiratory exchange of the membrane has also been determined and a corresponding absorption of $O_2$ (R.Q. assumed = 0·8) allowed for in the calculation of the diffusion experiment. In many cases the nitrogen samples only have been analysed for oxygen. The determination of a small percentage of nitrogen in oxygen is not nearly so accurate as the determination of a small percentage of oxygen in nitrogen, and as the diffusion of nitrogen is, moreover, of very little biological interest the determination was often omitted.

As an example I reproduce the protocol of one of the determinations.

Temp. during exp. 20·0°. Membrane: chitine from last dorsal segment of Oryctes larva preserved in spirit, washed with water. Thickness measured by means of screw micrometer divided in 0·01 mm. Measurements: 5·5, 6·0, 5·2, 5·1, 5·4, 5·7, 6·0. Average 5·6

Index correction 0·2

Thickness 54μ

| | | $O_2$ in nitrogen % | $N_2$ in oxygen % |
|---|---|---|---|
| Exp. begun 19th | 4.47 p.m. | 0·01 | 1·62 |
| Finished 20th | 10.52 a.m. | 0·59 | 1·99 |
| Difference | 1085 m. | 0·58 | 0·37 |

The volumes of the vessels are left 37·3 c.c., right 35·5 c.c. The volume of oxygen which has diffused into the nitrogen is therefore $\frac{0{\cdot}58}{100}\,37{\cdot}7$. It is reduced to 0° by multiplication with $\frac{273}{293}$. It could be reduced to normal dry pressure by multiplication with $\frac{B-f}{760}$, but in order to find the diffusion at a full atmosphere's pressure difference the result would have to be divided by $\frac{B-f}{760}$. All corrections for total pressure can therefore be omitted. The $O_2$ pressure difference between the two vessels has been at the beginning

$$\frac{100-(0{\cdot}01+1{\cdot}62)}{100}, \text{ at the end } \frac{100-(0{\cdot}59+1{\cdot}99)}{100},$$

or on an average $\frac{97{\cdot}9}{100}$. The result must be multiplied by $\frac{100}{97{\cdot}9}$ to get the diffusion at a full atmosphere's pressure difference, and we obtain the diffusion constant by further division by the time (1085 m.), multiplication by the thickness ($54\mu$) and division by the area of the membrane (0·794 sq. cm.) as follows:

$$\frac{0{\cdot}58}{100}\cdot 37{\cdot}7\cdot\frac{273}{293}\cdot\frac{100}{97{\cdot}9}\cdot\frac{1}{1085}\cdot\frac{54}{0{\cdot}794}=0{\cdot}013.$$

The reliability of this method was tested by means of determinations made on gelatine and on a rubber membrane. A membrane of gelatine which would stand the unavoidable pressure differences at the beginning and end of each experiment could not be prepared, but the difficulty was overcome by making metal plates fitting into the diffusion apparatus and provided with a number of small holes. Such a plate was immersed in a hot gelatine solution of about 15 p.c., which was allowed to set by cooling whereupon the plate was cut out and the gelatine removed on both sides by means of a razor. Two such plates were tested. One was $319\mu$ thick and had 36 holes of 0·930 mm. diameter. The total effective area was 0·245 sq. cm. The other of $380\mu$ thickness had 69 holes with a total area of 0·143 sq. cm. A series of determinations of the diffusion through these plates at 20° gave

| Plate | Duration m. | Diffusion constant | |
|---|---|---|---|
| | | $O_2$ | $N_2$ |
| I | 1009 | 0·301 | — |
| I | 1580 | 0·235 | 0·114 |
| II | 1622 | 0·294 | 0·214 |
| I | 731 | 0·286 | — |
| I | 362 | 0·296 | — |
| | Average | 0·28 | (0·16) |

The figure for oxygen is 82 p.c. of Hüfner's value for water. It agrees well with Hagenbach's general result that the diffusion through 20 p.c. gelatine is about 33 p.c. slower than through water, but is incompatible with Hagenbach's single determination of the diffusion of oxygen through gelatine which gave 1·6 as the value of the constant.

A series of determinations on a dry rubber membrane of 37$\mu$ thickness made at 20·8° gave

| Duration | Diffusion constant | |
|---|---|---|
| m. | $O_2$ | $N_2$ |
| 40 | 0·075 | — |
| 92 | 0·073 | — |
| 79 | 0·082 | 0·04 |
| Average | 0·077 | |

The average is somewhat higher than that obtained in the former series of experiments, viz. 0·064, but the temperature is 5° higher and Graham found that the diffusion of gases through rubber increases rapidly with the temperature.

After these tests which show that the method is on the whole reliable, though rather deviating results are occasionally met with, several series of experiments were made on membranes composed of animal tissues.

(*a*) *The influence of temperature upon the rate of diffusion.* Hüfner assumes in his calculation of the diffusion through the lungs of warm-blooded animals that the influence of an increased temperature is to diminish the rate of diffusion, because the diffusion is proportional to the coefficient of absorption, which decreases rapidly with increasing temperature, and is taken by him to be proportional further to the square root of the absolute temperature. It must be borne in mind, however, that the internal friction in water is greatly diminished by increasing temperature, and it is quite conceivable that this may cause an increase in the rate of diffusion of gas molecules. It is absolutely necessary therefore to study the problem experimentally.

The following series of determinations were made on peritoneal membrane from small dogs. The thickness of these membranes is sufficiently small (8–12$\mu$) to allow a determination to be made in about half-an-hour.

| I | | II | | III | |
|---|---|---|---|---|---|
| Tp. | Diff. const. $O_2$ | Tp. | Diff. const. $O_2$ | Tp. | Relative diff. rate* $O_2$ |
| 20·1° | 0·117 | 20·0° | 0·123 | 20° | 75 |
| 0·4° | 0·088 | 0·2° | 0·093 | 36° | 86·5 |
| 20·0° | 0·102 | 0·2° | 0·100 | 20° | 72 |
| 35·4° | 0·133 | 20·2° | 0·121 | 36° | 92 |
| 20·0° | 0·120 | 36·2° | 0·124 | — | — |

* The thickness of this membrane was not measured.

If these results are averaged and the diffusion at 20° taken as unity we find in the three series

| Tp. | I | II | III | Averages |
|---|---|---|---|---|
| 0·2°–0·5° | 0·78 | 0·76, 0·82 | — | 0·79±0·02 |
| 20° | 1·04, 0·91, 1·06 | 1·01, 0·99 | 0·98, 1·02 | 1·00±0·02 |
| 36° | 1·18 | 1·02 | 1·18, 1·26 | 1·16±0·05 |

The experiments show conclusively that the rate of diffusion of $O_2$ is increased with increasing temperature and an approximation to the rate of increase is obtained. According to Hüfner the diffusion rates for $O_2$ in water at the different temperatures should have been proportional to

|  | Abs. coeff. | $\sqrt{T}$ | Hüfner's relative diffusion rates |
|---|---|---|---|
| 0° | 0·0485 | 16·51 | 1·51 |
| 20° | 0·0310 | 17·11 | 1·00 |
| 36° | 0·0242 | 17·59 | 0·80 |

When these figures are divided by the relative figures for the internal friction of water at the same temperatures we get

|  | Calculated diffusion rates for $O_2$ in water | Observed diffusion rates for $O_2$ in conn. tissue |
|---|---|---|
| 0° | 0·83 | 0·79±0·02 |
| 20° | 1·00 | 1·00±0·02 |
| 36° | 1·12 | 1·16±0·05 |

The calculated figures agree with the observed within the possible errors of the latter and the results therefore lend some support to the assumption that the rate of diffusion is inversely proportional to the internal friction of water. On this assumption the diffusion constant could be calculated for any desired temperature. For practical purposes it is, however, safer and more convenient to utilise the actual determinations and to allow 1 p.c. increase or decrease in the diffusion constant for each degree the temperature is higher or lower than 20°. In the following all the determinations mentioned have been reduced to 20° in this way.

(*b*) *The diffusion of oxygen through connective tissue membranes of different thickness.* The following determinations have been made:

|  | Connective tissue from abdomen of frog |  |  | Peritonaeum of dog |  |
|---|---|---|---|---|---|
| Thickness | 63μ | 35μ | 17·5μ | 11·5μ | 7·65μ |
| Gas-blood | 0·106 | g.-g. 0·101 | g.-bl. 0·099 | g.-g. 0·120 | g.-g. 0·123 |
| Blood-blood | 0·118 | „ 0·120 | bl.-bl. 0·091 | „ 0·117 | „ 0·121 |
|  | — | „ 0·137 | — | „ 0·102 | „ 0·122 |
|  | — | — | — | „ 0·112 | „ 0·107 |
| — | — | — | — | „ 0·115 | — |
| Averages | 0·112 | 0·119 | 0·095 | 0·113 | 0·118 |

The general average is 0·113. It is obvious that there is no systematic difference between the results obtained with blood and those obtained with air and further that membranes of very different thickness give practically the same diffusion constant. If the invasion coefficient for oxygen as defined by Bohr(7) and "determined" by myself(8) were even approximately correct this ought not to be so.

The invasion coefficient for oxygen is defined by Bohr as the amount of oxygen which will penetrate in 1 minute into 1 sq. cm. of water surface when the $O_2$ pressure difference between the surface itself and the gas is 1 atmosphere. When determinations have to be made the gas pressure in the surface must be assumed to be equal to that in the interior of the fluid—that is the mixing must be absolutely complete and no diffusion whatever must take place. This condition cannot be fulfilled and it is moreover always very uncertain how near it has been approximated. I showed in 1910 that Bohr's determinations were at least six times too low and I pointed out that mine might also be too low and even much too low. My value was 0·076 c.c. or considerably lower than the diffusion constant. In the diffusion experiments according to the second method both invasion and evasion have to take place. When this is not taken into account the diffusion constant should be found too low, because the pressure head available for diffusion must be the difference between the total pressure and the amount necessary to bring about the invasion and evasion, which should, according to my determination of the invasion, correspond very nearly to the pressure necessary for the diffusion through a thickness of 3$\mu$. If such a correction were applied it is obvious that the diffusion determinations would become absurd, the thin membranes giving much higher values for the diffusion constant than the thick ones. In order to be at all compatible with the present determinations of the diffusion constant the invasion coefficient would have to be at least about five times higher than my determination in 1910.

I have made a renewed attempt to determine the invasion coefficient which it may be useful to record briefly, because it demonstrates clearly why such attempts are bound to fail. I employed the apparatus shown in Fig. 4. 1 is a smooth cylinder (diameter 13 mm., length 18 mm.) dipping into the trough 2 filled with a fluid into which the diffusion takes place. The cylinder can be revolved by means of the shaft 3 at a maximum rate of 4000 revolutions per minute. When revolving it will be covered with a film of fluid. The mixing arrangements in the trough and the rubber band 4 sliding against the cylinder secure a complete renewal of the film for each ⅓ revolution. Now let us suppose the fluid to be water at 37° and take it to form a film of only 1 $\mu$ thickness. The film of water being renewed 6000 times per minute 6 c.c. of water will pass over each sq. cm. of the cylinder surface per minute. If the water is free from dissolved gas and the apparatus revolves in an atmosphere of pure oxygen it should take up (according to my 1910 experiments) 0·076 c.c. $O_2$ per sq. cm. per minute, supposing the oxygen pressure in the film could be maintained approximately at 0. The absorption coefficient of $O_2$ in water at 37° being 0·024 this quantity would require 3 c.c. water for its solution at 1 atmosphere pressure. If 0·076 c.c. are actually taken up the $O_2$ tension difference between the gas and the whole of the water passing over the cylinder must be about ½ atmosphere and of course much smaller still between the actual surface of the water and the gas.

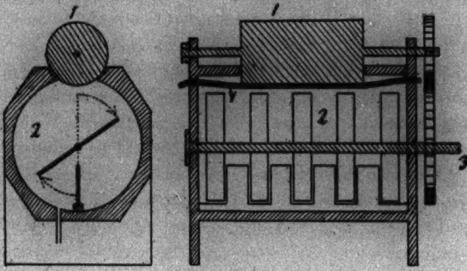


Fig. 4.

This means that the real invasion coefficient must be many times larger than any figure which could be found in experiments with water.

I have made a few experiments using completely reduced blood as the fluid. The invasion took place at room temperature and with a comparatively low oxygen pressure in the gas above the apparatus. After a suitable time the total quantity of oxygen taken up was determined by means of Barcroft's method. Assuming that the oxygen tension of the blood surface itself were not higher than that calculated for the total quantity of blood at the end of the experiment the "invasion coefficient" worked out as 0·3 or four times the earlier value. It is easy to see that this value must be a great deal too low. The oxygen molecules must diffuse a certain distance through plasma and through the walls of the red corpuscles before they can combine with the hæmoglobin. Supposing this distance to be on an average 1 $\mu$ the "invasion coefficient" found, which is about equal to the diffusion constant for water, would just represent the pressure difference necessary for the diffusion and the true invasion coefficient must be infinite. The average distance through which diffusion must in this case take place is perhaps less than $1\mu$, but it is obvious that as a determination of the invasion coefficient $\gamma$ the result ought to be written $0{\cdot}3 < \gamma < \infty$, the more so as the combination of $O_2$ with hgbl. is not instantaneous, but involves the existence of a certain $O_2$ pressure within the corpuscles during their passage across the cylinder.

**Bohr** maintained that in fluids in which the gas molecules were combined chemically in the very surface the process of absorption was different in character from a true invasion and much more rapid. I do not see why the process should be really different, but when chemical combination takes place the gas pressure in the surface can be maintained at a very low figure, though always above 0, considering the reaction time of the combination. I have made some experiments with potassium pyrogallate in the above apparatus. In this case the rate of "invasion" rose rapidly with increasing temperature, indicating in my opinion the increased rate of combination between oxygen and the solution. The maximum "invasion coefficient" obtained for this fluid at a temperature of 20° was about 12 which is, I suppose, still much below the real figure.

The processes of invasion and evasion of a gas must, as far as I can see, be similar to condensation and evaporation of a pure fluid. When such a fluid is in contact with an atmosphere saturated with its vapour evaporation and condensation balance each other, just as evasion and invasion must balance each other when tension equilibrium is maintained between a gas and a fluid. **Martin Knudsen** (9) has shown in the case of mercury that the rate of evaporation and consequently of condensation is so great that every molecule striking the surface of the fluid phase will be taken up. It seems to me very probable that this would hold also in the case of gas molecules striking a fluid in which they can become absorbed.

In any case invasion is a process which is so rapid that it can be left out of account altogether in dealing with the rate of absorption of gases in animal tissues.

(c) *The average diffusion constant for oxygen through connective tissue.* This at 20° is 0·113 and there does not seem to be any difference between the connective tissue obtained from frogs and that from mammals. The reason why the diffusion through connective tissue should be so much slower than through gelatine or water is unknown, but the fact that it is cannot, I think, be called in doubt. A single experiment has been made on goldbeater's skin of 23$\mu$ thickness which is connective tissue that has undergone some preparation. It shows a much lower diffusion constant, viz. 0·04. Still lower is the diffusion constant for chitin 0·013.

(*d*) *Determinations on the abdominal muscular wall of the frog.* The chief difficulty in this case is to obtain a reliable determination of the thickness of the tissue. In three cases, including the experiment according to method 1, I have measured the thickness by means of the arrangement described above. In one case I have made the measurement by means of a screw micrometer acting directly on the membrane, and in a fifth case I have cut out the membrane, determined the weight and calculated the thickness, assuming a specific gravity of 1·04. The results of the determinations, reduced to 20° and with two exceptions made at that temperature are:

| Thickness of membrane $\mu$ | Measured by | Diff. const. at 20 | |
|---|---|---|---|
| 140 | Screw micrometer | 0·116 | |
| 141 | Microscope | 0·145 | |
| 148 | — | 0·161 | |
| 221 | — | 0·122<br>0·122 | Experiment according to method 1 at 16° |
| 290 | Weighing | 0·131<br>0·136 | |
| | Average | 0·133 | |

The diffusion constant is unmistakably higher than for connective tissue though the difference is not considerable. When the fact is allowed for that the abdominal wall employed includes two layers of connective tissue of a total thickness averaging about 50$\mu$ the diffusion constant for oxygen through muscle must be higher still and works out as 0·139 or approximately 0·14. By means of this figure combined with measurements of the capillaries and their distribution in muscles it becomes possible to study a little more closely than hitherto the mechanism of the oxygen supply to muscles during rest and during work and it is chiefly with this purpose in view that the present investigation has been undertaken.

(*e*) *The relative diffusion constants of different gases.* Determinations were made with CO and $CO_2$ by the first method and with $N_2$ by the second, but these latter do not agree very well. Taking the diffusion constant of oxygen as a unity I have obtained the following results:

| Membrane | Thickness μ | $N_2$ | CO | $CO_2$ |
|---|---|---|---|---|
| Conn. tissue | 63 | — | 0·74 | 35·7 |
| „ | 17·5 | — | 0·76 | — |
| Muscle | 221 | — | 0·70 | — |
| „ | 290 | 0·70 | — | — |
| „ | 148 | 0·64 | — | — |
| „ | 140 | 0·45 | — | — |
| Conn. tissue | 35 | 0·52<br>0·49 | — | — |
| Peritonæum | 11·5 | 0·42<br>0·42 | — | — |
| Chitin | 54 | 0·6 | — | — |

When these results are averaged and compared with those obtained by other investigators I find:

|  | Relative diffusion constants |  |  |  |
|---|---|---|---|---|
|  | calculated for water | found by Hüfner for water | Exner soap solution | Krogh animal tissues |
| $N_2$ | 0·53 | 0·53 | 0·44 | 0·4–0·7 |
| CO | 0·89 | — | — | 0·73 |
| $O_2$ | 1 | 1 | 1 | 1 |
| $CO_2$ | 23·1 | 27·5 | 24·2 | 35·7 |

My figure for $CO_2$ is based on a single determination and may possibly be too high. The figure for CO which is, I believe, reliable is distinctly lower than the theoretical deduced for water. In the investigation of the rate of diffusion of gases through the alveolar wall by Marie Krogh (10) it is assumed that the different gases diffuse at the relative rates deduced theoretically for water and the results are based on determinations of the diffusion of carbonic oxide—this being the only gas available for the purpose. If the alveolar wall consists of ordinary connective tissue the diffusion constants calculated for oxygen through the lungs of man will have to be increased about 20 p.c. A direct determination of the relative rates of diffusion of $O_2$ and CO through the alveolar wall has been planned.

## SUMMARY.

Methods are described for measuring the rates of diffusion of gases through membranes of animal tissue and other substances.

The diffusion constant for a gas through a substance is defined as the number of c.c. (0°, 760 mm.) penetrating through 0·001 mm. (1μ) thickness and 1 sq. cm. surface per minute when the pressure difference is 1 atmosphere.

The diffusion constant for oxygen through animal tissues increases with increasing temperature—about 1 p.c. per degree, taking the rate at 20° as unity.

Diffusion of gases through animal tissues is much slower than through water or gelatine. The absolute diffusion constants for oxygen at 20° are:

| | | |
|---|---|---|
| Water ... ... | 0·34 | Hüfner |
| Gelatine ... ... | 0·28 | Krogh |
| Muscle ... ... | 0·14 | — |
| Connective tissue | 0·115 | — |
| Chitin ... ... | 0·013 | — |
| Indiarubber ... | 0·077 | — |

The "invasion coefficient" for oxygen into water is many times higher than the "determinations" hitherto made would indicate.

### REFERENCES.

(1) **Exner.** Annalen der Physik, 155. 1875.
(2) **Stefan.** Sitzber. d. Akad. d. Wiss. Wien, 77. Abth. II. 1878.
(3) **Hüfner.** Ann. d. Physik. u. Chem. Neue Folge, 60. 1897.
(4) **Hagenbach.** Ibid. 65. 1898.
(5) **Andresen.** Bioch. Ztschr. 74. 1916.
(6) **Graham.** Ann. d. Physik. 129. 1866.
(7) **Bohr.** Ann. d. Physik u. Chem. N. F. 68. 1899.
(8) **Krogh.** Skand. Arch. Physiol. 23. 1910.
(9) **Knudsen.** Danske Vid. Selsk. Oversigt. 1915.
(10) **Marie Krogh.** This Journal, 49. 1915.

# THE NUMBER AND DISTRIBUTION OF CAPILLARIES IN MUSCLES WITH CALCULATIONS OF THE OXYGEN PRESSURE HEAD NECESSARY FOR SUPPLYING THE TISSUE. BY AUGUST KROGH.

(*From the Laboratory of Zoophysiology, University of Copenhagen.*)

IN a preceding paper I have recorded measurements of the diffusion constants for certain gases and especially for oxygen in animal tissues. In order to utilise these determinations it is necessary to know further the rate at which oxygen is used up by the tissue in question and the average distance which an oxygen molecule has to travel from a capillary into the tissue before entering into chemical combination. In most tissues the arrangement of capillaries is too irregular to admit of detailed measurements and calculations but in the muscles we have a fairly regular distribution of capillary vessels, which are in the main parallel to the muscle fibres between which they are intercalated. I have therefore made the muscles the basis of my study.

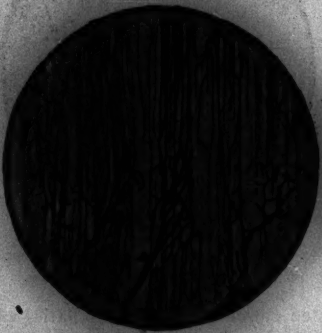


Fig. 1. Longitudinal section of injected M. gastrocnemius of the horse. × 70.

Fig. 2. Injected abdominal muscle wall of the guinea-pig. × 70.

The general arrangement of the vessels in striated muscles of mammals is as follows (Figs. 1 and 2). From small arteries, running in the main parallel to the fibres, arterioles are given off at more or less right angles and at rather constant intervals. These arterioles penetrate among the fibres, dividing freely, and each arteriole gives rise to two bundles of capillaries running along the fibres in opposite directions. In a muscle stretched to its normal length during rest the capillaries are more or less straight, but in a contracted muscle they become very sinuous; they anastomose to a certain extent forming long meshes about the fibres. From two bundles of capillaries meeting each other from opposite directions the blood is collected into small veins running like the arterioles mainly at right angles to the fibres and opening into larger veins following the corresponding arteries along the muscle fibres. The arrangement I have given above is similar to that figured by Ranvier[1] and by Kölliker[2].

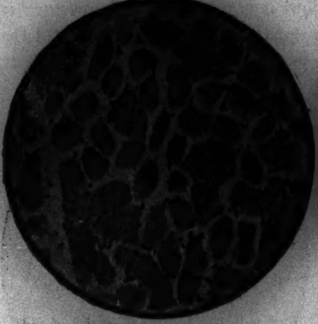


Fig. 3. Transverse section of the M. gastrocnemius of the horse. ×120.

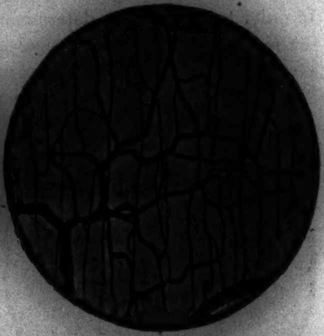


Fig. 4. Injected smooth muscle of the stomach of the frog. ×38. (Referred to later.)

Thin transverse sections of injected muscles show the capillaries as small coloured dots which are distributed with conspicuous regularity among the muscle fibres (Figs. 3 and 5), and it becomes at once apparent that no serious error can be committed by supposing each capillary to supply oxygen independently of all the others to a cylinder of tissue surrounding it. In a transverse section such a cylinder is represented by an area which can be taken as circular and the average area belonging to each capillary can be calculated from a counting of the number of capillaries in a transverse section by division of its total area with the number found.

[1] Ranvier. *Traité tech. d'Histologie*, fas. 4, p. 511. 1876.

[2] Kölliker. *Hdb. d. Gewebelehre*, 1. p. 380. 1889.

Supposing Fig. 6 to represent the cross-section of a capillary ($r$) with the cylinder of tissue ($R$) supplied by it we shall have oxygen molecules constantly leaving the capillary through the wall and entering the surrounding tissue where they will be used up at a rate determined by the gas exchange. The oxygen pressure difference between the capillary wall and a point at the distance $x$ from the centre of the capillary must be proportional to the gas exchange $p$ and inversely proportional to the diffusion rate $d$, and when these are known, together with the dimensions of the capillary and cylinder (their radii $r$ and $R$) and the distance $x$, it becomes possible to establish a mathematical formula from which the pressure difference can be calculated. The mathematician Mr K. Erlang has shown me the kindness to work out such a formula which runs

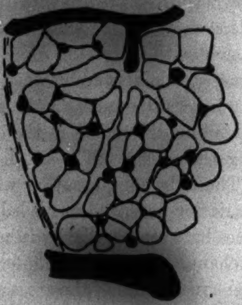


Fig. 5. Transverse section of injected muscle of the tongue of the cat. ×268.

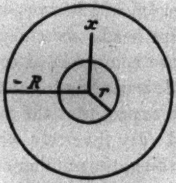


Fig. 6. See text.

$$T_0 - T_x = \frac{p}{d}\left(\tfrac{1}{2}R^2 \log_{\text{nat}}\frac{x}{r} - \frac{x^2 - r^2}{4}\right),$$

$T_0$ and $T_x$ being the oxygen tensions in the capillary and the point $x$ respectively. Putting $x = R$ and substituting ordinary logarithms for the natural we get the formula transformed to

$$T_0 - T_R = \frac{p}{d}\left(1{\cdot}15R^2 \log\frac{R}{r} - \frac{R^2 - r^2}{4}\right),$$

which will give us the maximum tension difference necessary to supply the muscle with oxygen. If this difference is found to be larger than the $O_2$ tension of the venous blood it follows that some portions at least of the muscle (near the venous ends of the capillaries) must suffer from want of oxygen, while if it is smaller the oxygen tension must be positive everywhere within the muscle.

In the above formula all distances are calculated in cm. $d$ is the rate of diffusion per minute through 1 cm.$^2$ and the distance of 1 cm. From the determinations given in the preceding paper we have for warm-blooded animals $d_{37} = 0{\cdot}164 \cdot 10^{-4}$ and for cold-blooded $d_{15} = 0{\cdot}133 \cdot 10^{-4}$. $p$ is the absorption of oxygen per cm.$^3$ per minute, and in the calculations given below I have taken the gas exchange of muscular tissue to be equal to that of the animal as a whole. $R$ is calculated from countings of capillaries on transverse sections of injected preparations, while $r$, which cannot safely be measured directly, can be taken provisionally to be determined by the size of the red corpuscles which have to pass through the capillaries. In most mammals the red corpuscles are not very different in size while those of frogs are much larger. It can be shown from the formula that $T_0 - T_R$ decreases but slowly with increasing $r$.

Between cold-blooded and warm-blooded animals and between warm-blooded of different size there are very large differences in the gas exchange ($p$) which must involve corresponding differences in the oxygen pressure heads necessary for supplying the muscles ($T_0 - T_R$), unless they are compensated in some way. The only compensation which is possible according to the formula is a variation of $R$, and one is led therefore to expect that the average distances between capillaries in muscles should vary inversely with the gas exchange or, what amounts to the same thing, the number of capillaries per square mm. of the cross-section should vary directly with the gas exchange.

I intended to test this deduction on a series of injection preparations of muscles from a number of different animals, but I have had to reduce the programme very considerably on account of the unexpected difficulties met with in obtaining complete injections of muscular tissue. Injections from the aorta or any large arteries, which produce excellent results in most organs, have invariably failed in the case of muscles though the technique was varied in several ways. The vessels were washed out carefully with saline at body temperature and nitrites were added to produce a vaso-dilatation. In some experiments on the cod the muscles were tetanised at intervals before and during the injections, but though more capillaries became filled than without tetanisation the injections were far from complete. The best results have been secured by injecting directly through a muscle artery, tying as far as possible all the arterial anastomoses and employing a high pressure. When practicable I have finally tied the vein and set up a very considerable pressure before tying the artery. I believe that the material to be injected is best left for a day or two after killing the animal before the injection is made.

As injection fluid I have used gelatine stained with freshly prepared Prussian blue or with Indian ink.

I have obtained good injections of the following muscles: M. gastrocnemius of a horse[1], M. semimembranosus of a dog[1], M. masseter of a guinea-pig, Mm. sartorius and submaxillaris of a frog. Certain portions of a prætracheal muscle of the guinea-pig and of an eye muscle of a cod are also probably completely injected.

The completeness or otherwise of the injection can practically always be judged from longitudinal sections and in certain cases, especially in the cod and frog, the capillaries have been counted on thick longitudinal sections by taking optical transverse sections of them. This has been done as follows: A micrometer in the eyepiece of the microscope is arranged at right angles to the muscle fibres and capillaries, the surface of the section is focussed and the position of the distinctly visible capillaries marked on squared paper. The microscope tube is lowered a certain small amount (usually five scale divisions corresponding to 4·5$\mu$ absolutely or, when the section is mounted in balsam of 1·5 refractive index, to 6·75$\mu$). The capillaries which thereby become visible are put into the diagram and the process repeated through the whole thickness of the section or to such a depth as is compatible with obtaining clear images of the capillaries.

In most cases countings have been made on actual transverse sections by means of a micrometer ruled in squares. I give as an example the countings made on five different transverse sections from the gastrocnemius muscle of the horse.

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | 45 | 34 | 38 | 38 | 31 |
| | 40 | 34 | 42 | 43 | 33 |
| | 42 | 40 | 43 | 47 | 43 |
| | 41 | 46 | 41 | 49 | 39 |
| | 44 | 44 | 46 | 33 | 36 |
| | 36 | 41 | — | — | — |
| | 49 | 38 | — | — | — |
| Average | 42 | 39 | 42 | 42 | 36 |

The general average is 40·5 and the dispersion (standard deviation) of the single countings ± 5, which shows the regularity of the distribution. As the area of the square counted is 0·0300 mm.$^2$ the result of one counting corresponds to 1350 ± 170 (12·4 p.c.) and the general result to 1350 ± 31 capillaries per square millimetre.

---

[1] For the material for these and kind help in carrying out the preparation and injection I am indebted to Prof. Paulli of the Veterinary High School, Copenhagen.

In like manner the number of capillaries per mm.$^2$ of the dog's semimembranosus has been determined from 30 countings to 2630 ± 51 with a dispersion of ± 280 (10·6 p.c.) and the number in the masseter muscle of the guinea-pig from nine countings to 2840 ± 100 with a dispersion of 300 (10·6 p.c.). From the prætracheal muscle of the guinea-pig only three countings of an area of 0·0150 mm.$^2$ have been obtained giving the results 55, 55 and 58 capillaries corresponding to 3700 per mm.$^2$ In the muscles of frogs and a cod I have found from 300 to 500 capillaries per mm.$^2$.

It is of course impossible to draw valid conclusions from such a small number of determinations, but as far as they go the countings would seem to indicate that the capillary network is closer in warm-blooded than in cold-blooded animals and closer in small mammals than in larger forms. With a reliable injection technique it would certainly be worth while to count the number of capillaries on transverse sections of various muscles from a larger number of vertebrates.

The results of the counting and other necessary data have been put together in a small table and the resulting maximum oxygen tension difference ($T_0 - T_R$) between the blood and the muscular tissue deduced. By the formula $T_0 - T_R$ is expressed in atmospheres. In the table I have reduced the figures to mm. mercury pressure.

| | Weight kg. | Metabolism cal. per kg. and hour | Number of capillaries per mm.$^2$ of striped muscle | $R$ $\mu$ | Diam. of red corpuscles $2r$ $\mu$ | $T_0 - T_R$ mm. Hg. |
|---|---|---|---|---|---|---|
| Cod | 1 | 0·4 | 400 | 28 | 8·5 | 0·4 |
| Frog | 0·04 | 0·4 | 400 | 28 | 15 | 0·25 |
| Horse | 500 | 0·5 | 1400 | 15 | 5·5 | 0·1 |
| Dog | 5 | 3 | 2500 | 11·3 | 7·2 | 0·2 |
| Guinea-pig | 0·5 | 6 | 3000 | 10·3 | 7·2 | 0·3 |

The table shows that the oxygen pressure head necessary for supplying the muscles with oxygen is remarkably small, so small indeed that even during the heaviest muscular work, which may possibly increase the metabolism twenty-fold, the oxygen tension of the muscular tissue will be practically equal to that of the venous blood, the maximum difference in the case of the guinea-pig being only 6 mm. or less than 1 p.c. of an atmosphere.

In other tissues than muscles the arrangement of the capillaries is not sufficiently regular to allow direct calculations to be made but a simple inspection of injection preparations is sufficient to show that in most glands and in the central nervous system the capillary network is even closer than in striated muscles. In smooth muscles (Fig. 4 *supra*) and in connective tissue it is more open, but we have every reason to suppose that here also the oxygen consumption is much lower, and it would seem reasonable to conclude therefore from the measurements and calculations that there is in the tissues of all organs a positive oxygen pressure equal to or even higher than that of the venous blood leaving each organ. For reasons which will be fully discussed in the succeeding paper such a conclusion is not warranted.

## SUMMARY.

In striated muscles the capillaries are arranged with such regularity along the muscle fibres that each capillary can be taken to supply a definite cylinder of tissue the average cross-section of which can be determined by counting the capillaries in a known area of the transverse section.

A formula is given which allows the calculation of the oxygen pressure head which is necessary and sufficient to supply the muscle with oxygen from the capillaries.

The number of capillaries per square mm. of the transverse section of striated muscle appears to be a function of the intensity of the metabolism, being higher in small mammals than in larger forms.

The necessary oxygen pressure head deduced from the total number of capillaries is in all cases extremely low. |||

# INFLUENCE OF SAPROPHYTE BACTERIA ON OXIDATION IN HIGHER ANIMALS. (5th Series of experiments.)

By I. KIANIZIN, *Professor in the University of Odessa.*

In earlier papers[1] I have given an account of experiments which show that prolonged life is impossible in the absence of saphrophyte bacteria, since in their absence the formation of oxidising ferments is so much reduced that auto-intoxication takes place. Bacteria enter the body partly in the food and partly in the inspired air. It was desirable then to determine how far the sterilisation of the air would reduce oxidation in the body and the amount of catalases in the blood. To this end I have made some further experiments. At the same time I have determined the quantity of $NH_3$, amino-acids ($NH_2$), urea, phosphoric and sulphuric acid.

The catalase content of the blood was determined by the ordinary methods, *i.e.* by taking blood from the vein in a rabbit's ear. After having let it stand, 0·5 c.c. of serum was taken and diluted up to 10 c.c. of physiological solution. A portion of this was boiled in order to kill ferments; after cooling 5 c.c. of 2 p.c. oxygenated water ($H_2O_2$) were added to both portions and these were mixed together in a thermostat at a temperature of 37° (for 15 minutes); after having added water to the mixture so as to produce 100 c.c. and after adding to it 15 c.c. of sulphuric acid (25 p.c.), it was standardized at $\frac{1}{20}$ by means of normal $MnKO_4$. There was always an important difference between the portion containing the killed ferments and the portion containing the non-killed ones, and that difference was calculated in proportion to the $H_2O_2$ (1 c.c. of $\frac{1}{20}$ normal solution $MnKO_4 = 0{\cdot}00085\ H_2O_2$). The quantity of ammonia and of amino-acids ($NH_2$) was determined according to Sörensen's method modified by **Matthison**; the quantity of $NH_3$ was determined by **Folin**'s method and the uric acid by Hopkins' method modified by **Folin** and **Schaffer**; the total N in the urine, excrements and food by Kjeldal's method; the N of the urea by Prof. Borodin's method; the precipitated leucomaines by Scheibler's method; the hydrocarbons were analysed by Marker's method.

---

[1] Cf. This *Journal*, 50. p. 391, 1916, for a summary of the observations of myself and of others.

In this work, on account of the irregularity in the working of water and air pumps, instead of ventilating the apparatus (which was hermetically sealed) by means of an exhaust pump, I used a compressing air pump worked by a motor (Heiss luft motor). This motor compressed atmospheric air first in a spiral metallic tube (1·5 cm. in diameter and 1½ metre long) heated to a temperature of over 160° C.; afterwards the air was expelled for a more thorough sterilisation in a metal vessel hermetically sealed with double walls (the space between the walls being about 5 litres), where the air was heated to 90° C. (with a ventilation speed from 3 to 4·5 litres per minute); then the hot air was directed by means of a glass tube into a bottle of about 10 litres content, into the bottom of which had been poured a solution of corrosive sublimate (1 : 500) to cool and moisten the air; then the air was filtered through a glass tube (5 cm. in diameter and 20 cm. in length), well stoppered with sterilised hygroscopic cotton-wool, and lastly the air, in which all organised life had been killed, was introduced through a glass tube into d'Arsonval's apparatus, cooled down to the temperature of the room and entirely sterilised (controlled by bouillon). d'Arsonval's apparatus was sterilised in the following manner: the metallic parts by high temperature, the glass parts by sublimate, the skin and hair of the animal were sterilised by sublimate (1 : 500) and washed in several litres of sterilised water. The impermeability of the apparatus to air was controlled by means of a manometer. The ventilation speed was from 3 to 4·5 litres per minute; the volume of the apparatus being of about 50 litres, the air was renewed in it from three to five times per hour. With this arrangement there was large reduction in the access of saprophytes to the animal organism. The results are given in Table I.

It will be seen that in the animals supplied with sterile air, there was a great and constant change in Robin's coefficient, *i.e.* that the oxidising processes were much reduced. The reduction can only be explained by the diminution in the access of bacteria. The investigations of Portier and of Slovtzoff have shown that leucocytes contain oxidising ferments. These oxidising ferments are I hold formed by the leucocytes in the process of digesting bacteria.

The results also show that there is an increase of catalase in the blood notwithstanding a decrease in the oxidising processes. This supports the view that catalases are not oxidising ferments. Their function is to regulate oxidation.

| N | Animal and conditions of the exp. | Duration of the exp. in days | Weight (before) | Weight (after) | Mean weight | N in the food | Carbohydrates in the food | N in the excrements | Carbohydrates in the excrements | Assimilation N | Assimilation carbohydrates | Total N in the urine to 1 kilo and 1 day | N of urea to 1 kilo and 1 day | N of leucomain to 1 kilo and 1 day | NH$_3$ to 1 kilo and 1 day | NH$_2$ (amino-acids) to 1 kilo and 1 day | Uric acid to 1 kilo and 1 day | SO$_3$ to 1 kilo and 1 day | P$_2$O$_5$ to 1 kilo and 1 day | Quantity of catalase, calculated in proportion to the H$_2$O$_2$ | Coefficient of A. Robin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♂ | g | r | a | m | m | g | r | % | % | a | m | m | g | r | a | m | m | | |
| 1 | Rabbit conditions normal | 3 | 1628 | 1583 | 1605 | — | — | — | — | — | — | 0·193 | 0·139 | — | — | — | — | — | — | — | 100 : 72·0 |
| 2 | | 4 | 1118 | 1098 | 1108 | — | — | — | — | — | — | 0·152 | 0·097 | 0·029 | 0·009 | 0·007 | 0·008 | 0·0097 | 0·072 | 0·00204 | 100 : 63·8 |
| 3 | | 5 | 1260 | 1243 | 1251 | — | — | — | — | — | — | 0·335 | 0·231 | 0·034 | 0·009 | 0·006 | 0·003 | — | — | 0·04165 | 100 : 68·9 |
| 4 | | 6 | 1325 | 1253 | 1289 | — | — | — | — | — | — | 0·202 | 0·134 | 0·008 | 0·009 | 0·007 | 0·009 | 0·0099 | 0·072 | 0·04080 | 100 : 73·9 |
| 5 | | 4 | 1465 | 1369 | 1417 | — | — | — | — | — | — | 0·288 | 0·201 | 0·023 | 0·005 | 0·010 | 0·008 | 0·0089 | 0·118 | 0·03655 | 100 : 70·0 |
| 6 | | 4 | 1270 | 1263 | 1266 | 2·147 | 84·1 | 0·521 | 9·172 | 75·8 | 89·1 | 0·517 | 0·374 | 0·049 | 0·029 | 0·030 | 0·016 | 0·0014 | 0·028 | 0·03865 | 100 : 72·3 |
| 7 | | 4 | 1198 | 1095 | 1146 | 1·707 | 69·14 | 0·365 | 6·578 | 78·6 | 90·5 | 0·473 | 0·355 | 0·033 | 0·022 | 0·027 | 0·007 | 0·0078 | 0·202 | 0·04250 | 100 : 72·9 |
| 8 | | 3 | 1065 | 1123 | 1094 | 1·852 | 73·70 | 0·264 | 6·117 | 80·3 | 91·7 | 0·170 | 0·119 | 0·014 | 0·014 | 0·011 | 0·008 | 0·009 | 0·080 | 0·03825 | 100 : 70·2 |
| | | | | | | | | Average | | 78·2 | 90·4 | | | | | | | | | | |
| 9 | Rabbit sterilisation | 4* | 1278 | 1287 | 1284 | — | — | — | — | — | — | 0·309 | 0·151 | 0·058 | 0·056 | 0·036 | 0·016 | — | — | — | 100 : 48·8 |
| 10 | | 4 | 1193 | 1157 | 1175 | — | — | — | — | — | — | 0·268 | 0·139 | 0·024 | 0·108 | 0·024 | 0·015 | 0·021 | 0·149 | 0·12750 | 100 : 52·0 |
| 11 | | *6† | 1244 | 1143 | 1193 | 2·273 | 90·8 | 0·536 | 13·0 | 76·4 | 85·6 | 0·497 | 0·312 | 0·070 | 0·049 | 0·021 | 0·003 | 0·027 | 0·212 | 0·09605 | 100 : 62·7 |
| 12 | | 3 | 1133 | 1131 | 1132 | 0·892 | 35·6 | 0·176 | 5·58 | 80·2 | 84·3 | 0·588 | 0·360 | 0·059 | 0·048 | 0·028 | 0·024 | 0·038 | 0·206 | 0·05780 | 100 : 61·2 |
| 13 | | 4 | 1283 | 1253 | 1268 | 1·852 | 73·7 | 0·438 | 16·4 | 76·3 | 77·8 | 0·457 | 0·250 | 0·053 | 0·030 | 0·028 | 0·024 | 0·036 | 0·204 | 0·06460 | 100 : 54·7 |
| 14 | | 4 | 1253 | 1203 | 1228 | 1·515 | 54·8 | 0·325 | 10·7 | 78·5 | 81·3 | 0·319 | 0·191 | 0·055 | 0·041 | 0·038 | 0·009 | 0·029 | 0·122 | 0·06800 | 100 : 59·9 |
| | | | | | | | | Average | | 77·8 | 82·2 | | | | | | | | | | |

* In experiments 9 and 11 the animals died.

† The urine during 5 days

The quantity of leucomaines, $NH_3$, of amino-acids, of phosphoric and sulphuric acids in the urine of animals in sterilised conditions increases, and the quantity of uric acid remains without any noticeable change.

The noxious influence on nutrition of air in closed rooms, of air overheated by certain systems of central heating, and the beneficial effect of life out of doors are readily explained by the theory I have given.

# THE RESPIRATORY RESPONSE TO ANOXÆMIA.

By J. S. HALDANE, M.D., F.R.S., J. C. MEAKINS, M.D., Lt.-Col., C.A.M.C. and J. G. PRIESTLEY, B.M., M.C., Capt. R.A.M.C.

(*From the Clinical Laboratories No.* 15 *Canadian General Hospital, Taplow and Ashhurst War Hospital, Littlemore, Oxford.*)

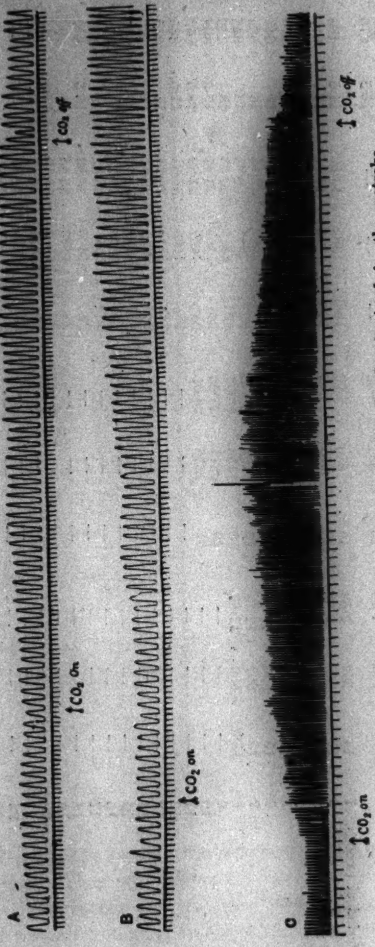
It has been recognised for some time that ordinarily the respiration is regulated with the utmost delicacy in correspondence with the $CO_2$ tension of the blood passing through the respiratory centre[1] and it is now generally agreed upon that what the centre actually responds to is increased hydrogen ion concentration.

Fig. 1 shows quantitative records of the breathing taken by means of an apparatus which will be described in the subsequent paper on shallow breathing. In each case the subject of the experiment was sitting at rest while breathing from the apparatus. The tracings show the records obtained when breathing air containing (*a*) 3·28 p.c., (*b*) 4·29 p.c. $CO_2$. Study of these tracings brings out clearly the following points. Firstly the characteristic effect of $CO_2$ is increased in depth of respiration accompanied by only slight quickening of the rate. In this connection we may refer to the well known "air hunger" of diabetic coma. Here also the deep protracted breaths constitute the response of the respiratory centre to increased hydrogen ion concentration in the arterial blood caused by the state of "acidosis." Secondly it also appears that when the breathing of air containing added $CO_2$ had been continued for some time the depth of the respirations and the total ventilation of the lungs per minute showed a tendency to diminish. For example in the tracing taken when breathing 4·29 p.c. of $CO_2$ the depth of respiration and ventilation per minute steadily increase for about seven minutes and then progressively fall off again. The fall is probably connected with fatigue and perhaps also with the process of adaptation discovered by Yandell Henderson[2], who found that the $CO_2$ capacity of the blood of dogs increased when they were made to breathe air containing an added amount of $CO_2$. Thirdly it is evident from the tracings that as long as the breathing of the $CO_2$ is continued there is a definite, though not very great, increase in the rate of respiration, which shows no tendency to diminish with the decreasing volume.

---

[1] Haldane and Priestley. This *Journal*, **32**. p. 225. 1905.

[2] Henderson and Haggard, *Journ. of Biol. Chem.* **33**. p. 333. 1918.



This and subsequent curves are read from left to right. Inspiration being the upstroke.

Fig. 1. Subject J. S. H.

(a) Breathing 3·28 p.c. $CO_2$. Time marker = 2 seconds,
(b) „ 4·29 p.c. $CO_2$. Time marker = 2 seconds,
(c) „ 4·29 p.c. $CO_2$. Time marker = 10 seconds.

Table I gives the numerical data deduced from the tracings.

TABLE I.

| Subject | | J. S. H. | | | J. S. H. | | | J. S. H. | | | J. G. P. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mixture breathed | | Air + 3·28 % $CO_2$ | | | Air + 4·29 % $CO_2$ | | | Air + 4·29 % $CO_2$ | | | Air + 4·38 % $CO_2$ | | |
| | Time in succ. half mins. | Rate of resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate of resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate of resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate of resp. per min. | Vol. per resp. c.c. | Vol. per min. litres |
| Prelim. control period breathing air | — | — | — | — | — | — | — | 18 | 563 | 10·12 | 15·5 | 344 | 5·34 |
| | — | 14 | 534 | 7·47 | — | — | — | 18 | 555 | 9·98 | 14 | 351 | 4·91 |
| | — | 14 | 543 | 7·60 | 15 | 523 | 7·85 | 18 | 550 | 9·90 | 15 | 398 | 5·97 |
| | — | 14 | 568 | 7·96 | 15·5 | 537 | 8·32 | — | — | — | 16·5 | 376 | 6·20 |
| Period of experiment breathing mixture | 1 | 14 | 559 | 7·83 | 15·5 | 510 | 7·90 | 19·5 | 682 | 13·30 | 14·5 | 463 | 6·71 |
| | 2 | 15 | 640 | 9·60 | 15 | 541 | 8·12 | 16 | 921 | 14·74 | 16 | 617 | 9·87 |
| | 3 | 14·5 | 747 | 10·84 | 16 | 684 | 10·95 | 21 | 983 | 20·66 | 17 | 767 | 13·04 |
| | 4 | 15·5 | 746 | 11·56 | 16 | 733 | 11·73 | 22 | 1003 | 22·03 | 18·5 | 879 | 16·26 |
| | 5 | 15·0 | 803 | 12·04 | 18 | 877 | 15·78 | 22 | 1229 | 27·06 | 19 | 648 | 12·31 |
| | 6 | 15 | 856 | 12·84 | 17 | 1024 | 17·41 | 23 | 1265 | 29·11 | 18 | 635 | 11·43 |
| | 7 | 15 | 850 | 12·75 | 17 | 1194 | 20·30 | 24 | 1288 | 30·91 | 18 | 628 | 11·30 |
| | 8 | 15·5 | 830 | 12·87 | 18 | 1153 | 20·77 | 23 | 1319 | 30·33 | 18 | 720 | 12·96 |
| | 9 | — | — | — | 19 | 1211 | 23·03 | 24 | 1386 | 33·27 | 17·5 | 608 | 10·64 |
| | 10 | — | — | — | 21 | 1238 | 26·01 | 24 | 1457 | 34·99 | 18·5 | 662 | 12·25 |
| | 11 | — | — | — | — | — | — | 25 | 1464 | 36·61 | 17·5 | 885 | 15·49 |
| | 12 | — | — | — | — | — | — | 24 | 1511 | 36·28 | 18 | 675 | 12·15 |
| | 13 | — | — | — | — | — | — | 24 | 1655 | 39·71 | 18 | 760 | 13·68 |
| | 14 | — | — | — | — | — | — | 23 | 1511 | 34·77 | 17 | 727 | 12·36 |
| | 15 | — | — | — | — | — | — | 24 | 1655 | 39·71 | 16 | 776 | 12·42 |
| | 16 | — | — | — | — | — | — | 22 | 1614 | 35·52 | 18 | 836 | 15·05 |
| | 17 | — | — | — | — | — | — | 23 | 1605 | 36·93 | 19·5 | 847 | 16·52 |
| | 18 | — | — | — | — | — | — | 24 | 1493 | 35·85 | 18 | 796 | 14·33 |
| | 19 | — | — | — | — | — | — | 24 | 1462 | 35·10 | 17·5 | 733 | 12·83 |
| | 20 | — | — | — | — | — | — | 24 | 1444 | 34·67 | — | — | — |
| | 21 | — | — | — | — | — | — | 25 | 1315 | 32·87 | — | — | — |
| | 22 | — | — | — | — | — | — | 24 | 1243 | 29·84 | — | — | — |
| | 23 | — | — | — | — | — | — | 25 | 1207 | 30·19 | — | — | — |
| | 24 | — | — | — | — | — | — | 24 | 1162 | 27·91 | — | — | — |
| | 25 | — | — | — | — | — | — | 24 | 1100 | 26·50 | — | — | — |
| | 26 | — | — | — | — | — | — | 24 | 1055 | 25·33 | — | — | — |
| | 27 | — | — | — | — | — | — | 25 | 970 | 24·26 | — | — | — |

So far we have only considered the effects of altering the $CO_2$ percentage of the air breathed. It next becomes of interest to study the effects, if any, of altering the proportion of oxygen in the inspired air.

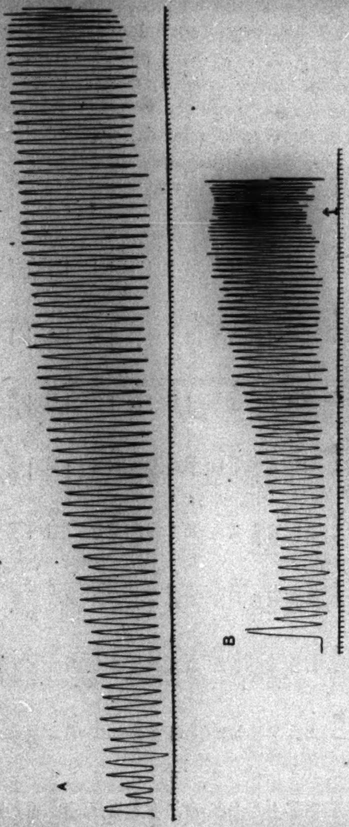


Fig. 2. Subject Cpl. M.

(a) Rebreathing—Concertina filled with oxygen—$CO_2$ accumulating.
(b) " " " air " "
Time marker = 2 seconds. Arrow shows point where lips were distinctly blue.

Fig. 2 shows the records of a subject rebreathing from the recording "concertina." Thus only about 4 litres of air were available and the rise of $CO_2$ percentage and the fall of oxygen percentage were rapid—with such a small volume of air the oxygen percentage falls more quickly

## TABLE II.

| Name | | Initial mixture | Rates and volumes at successive half minutes | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Capt. F. | Rate per min. | Air | 12 | 12 | 12 | 14 | 18 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 12·5 | 10 | 10 | 12 | 12 | 14 | 14 | 17 | 17 | — | — | — | — | — | — |
| | C.c. per resp. | Air | 670 | 960 | 1150 | 1290 | 1610 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 740 | 820 | 930 | 870 | 1000 | 1340 | 1310 | 1350 | 1700 | — | — | — | — | — | — |
| | Litres per min. | Air | 8·05 | 11·49 | 13·77 | 18·07 | 29·02 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 9·21 | 8·23 | 9·26 | 10·46 | 12·04 | 18·74 | 18·34 | 22·99 | 28·89 | — | — | — | — | — | — |
| Capt. McC. | Rate per min. | Air | 10 | 14 | 16 | 22 | 28 | 32 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 12 | 11 | 11 | 12 | 12 | 13 | 14 | 15 | 16 | 19 | 21 | 22 | 24 | 24 | 25 |
| | C.c. per resp. | Air | 1410 | 1370 | 1470 | 1850 | 2200 | 2790 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 1170 | 1120 | 980 | 1180 | 1190 | 1510 | 1550 | 1510 | 1830 | 1850 | 1710 | 1990 | 2140 | 2380 | 2370 |
| | Litres per min. | Air | 14·13 | 19·23 | 23·43 | 40·7 | 61·7 | 89·4 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 14·0 | 12·3 | 10·7 | 14·2 | 14·3 | 19·7 | 21·6 | 22·7 | 29·3 | 35·1 | 36·0 | 43·8 | 51·4 | 57·2 | 59·3 |
| Corp. M. | Rate per min. | Air | 16 | 17 | 18 | 23 | 29 | 48 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 14 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 18 | — | — | — | — | — |
| | C.c. per resp. | Air | 1090 | 1440 | 1820 | 1930 | 2510 | 2240 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 1260 | 1300 | 1830 | 1880 | 2490 | 2330 | 2880 | 2710 | 2990 | 2780 | — | — | — | — | — |
| | Litres per min. | Air | 17·4 | 25·2 | 32·8 | 44·4 | 72·7 | 107·7 | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 17·7 | 19·5 | 27·4 | 28·1 | 37·3 | 34·9 | 43·2 | 40·6 | 44·9 | 50·0 | — | — | — | — | — |
| Capt. V. | Rate per min. | Air | 20 | 22 | 20 | 23 | 29 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 22 | 24 | 22 | 24 | 26 | 29 | 30 | — | — | — | — | — | — | — | — |
| | C.c. per resp. | Air | 560 | 870 | 1610 | 2560 | 3060 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 690 | 750 | 1270 | 2070 | 2200 | 2390 | 2310 | — | — | — | — | — | — | — | — |
| | Litres per min. | Air | 11·3 | 19·2 | 32·2 | 58·9 | 88·6 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 15·1 | 18·0 | 27·9 | 49·6 | 57·3 | 71·3 | 69·4 | — | — | — | — | — | — | — | — |
| Capt. P. | Rate per min. | Air | 28 | 29 | 32 | — | — | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 19·5 | 20·5 | 19·5 | 21·5 | 26 | 29 | — | — | — | — | — | — | — | — | — |
| | C.c. per resp. | Air | 920 | 2000 | 3210 | — | — | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 540 | 850 | 970 | 1490 | 1950 | 2730 | — | — | — | — | — | — | — | — | — |
| | Litres per min. | Air | 25·8 | 57·9 | 102·8 | — | — | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 10·6 | 17·5 | 19·0 | 32·1 | 50·8 | 79·2 | — | — | — | — | — | — | — | — | — |
| J. S. H. | Rate per min. | Air | 14 | 16 | 24 | 48 | 66 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 14 | 14 | 16 | 18 | 20 | 25 | 30 | — | — | — | — | — | — | — | — |
| | C.c. per resp. | Air | 1000 | 1400 | 1850 | 1970 | 1980 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 880 | 1220 | 1720 | 2210 | 2460 | 2680 | 2690 | — | — | — | — | — | — | — | — |
| | Litres per min. | Air | 14·0 | 22·3 | 44·4 | 94·3 | 130·7 | — | — | — | — | — | — | — | — | — | — |
| | | Oxygen | 12·3 | 17·1 | 27·5 | 39·8 | 49·2 | 67·1 | 80·6 | — | — | — | — | — | — | — | — |

than the $CO_2$ percentage rises. The reason of this temporary inequality between the amount of oxygen used up and the amount of $CO_2$ given out is to be found in the fact that the body possesses very considerable storage capacity for $CO_2$ while it has practically none for oxygen.

Two series of records were taken with the concertina filled with (*a*) air, (*b*) oxygen at the start.

Table II gives the figures deduced from this and similar tracings.

They show (1) the effect of want of oxygen in lowering the threshold for $CO_2$, and (2) that on the whole the result of low oxygen percentage in the air inspired is to cause ultimately quickening of the rate and diminution of the depth of respiration.

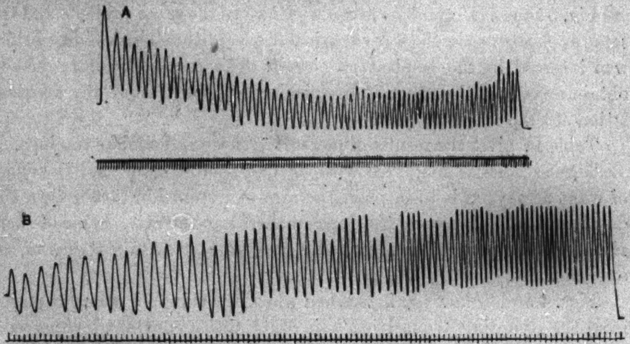


Fig. 3. Rebreathing through soda-lime from concertina. Time marker = 2 seconds. (*a*) Subject Cpl. M. (*b*) Subject J. S. H.

Records were obtained by breathing in and out of a large galvanised iron cylinder attached to a different recording apparatus. Tracings were taken (*a*) when the cylinder was filled with almost pure oxygen to start with; (*b*) when the cylinder was filled with air. In each case the breathing is affected by the gradually increasing proportion of $CO_2$ in the air inspired but in the one case the oxygen supply remains at least equal to ordinary air throughout the experiment while in the other case the oxygen percentage is continually decreasing below the normal. The fall however is not enough to produce any marked anoxæmia. On measuring up the tracings it is found that the effect of the diminishing oxygen percentage is to lower the threshold of the respiratory centre for $CO_2$, and consequently the depth of each respiration and the ventilation per minute increase more rapidly when the cylinder contains air than when it is filled with oxygen at the beginning of the experiment.

The measurements of the tracings are given in Table III.

TABLE III.

| Time in successive 2 min. periods | Air |  |  | Oxygen |  |  |
|---|---|---|---|---|---|---|
|  | Rate per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate per min. | Vol. per resp. c.c. | Vol. per min. litres |
| 1st | 13 | 1460 | 18·9 | 13 | 1540 | 19·6 |
| 2nd | 14 | 1940 | 27·1 | 13 | 2090 | 29·2 |
| 3rd | 14 | 2540 | 35·0 | 14 | 2310 | 32·9 |
| 4th | 14 | 3100 | 43·3 | 16 | 2320 | 36·5 |
| 5th | 14 | 4120 | 57·7 | 15 | 2810 | 42·2 |
|  | — | — | — | 17 | 3610 | 60·2 |

Fig. 3 shows the tracings taken with two of the same subjects and the same apparatus as in the last experiment except that a soda lime purifier was inserted in the respiratory circuit. The result therefore was to obtain records of the effect of diminishing oxygen without any accumulation of $CO_2$.

Table IV gives the results of measuring these and similar tracings.

These results as compared with the $CO_2$ results, show that the effect of want of oxygen is to increase the rate of respiration rather than the depth, while $CO_2$ increases the depth rather than the rate. Some of them also show the production of periodic breathing at certain stages of the oxygen want.

Figs. 4 and 5 show tracings taken when breathing for a longer period from the recording "concertina" air containing a constant low percentage of oxygen.

Table V gives the numerical data corresponding to these and similar tracings.

These records differ remarkably from the records of the effect of increased $CO_2$. It will be seen that the first result of diminution in the oxygen percentage is increase in the depth of respiration owing to lowering of the threshold exciting value of $CO_2$[1]. There then comes a point at which the breathing becomes periodic. The subject of periodic breathing was fully investigated by **Haldane** and **Douglas**[2]. They proved that the essential cause of the periodicity is the much quicker action of want of oxygen as compared with that of increase of $CO_2$. Owing to the great solubility of $CO_2$ in the body fluids changes of amount of $CO_2$ only slowly produce effective changes of tension. The result of

---

[1] **Haldane** and **Poulton**. This *Journal*, 37. p. 404. 1908.

[2] **Haldane** and **Douglas**. *Ibid.* 38, p. 401. 1909.

TABLE IV.

| Name | | Rates and volumes in consecutive half minutes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J. G. P. | Rate per min. | 18 | 16 | 16 | 16 | 16 | 15 | 16 | 18 | 22 | 34 | — | — |
| | Vol. per resp., c.c. | 430 | 440 | 505 | 580 | 645 | 750 | 795 | 835 | 1195 | 1415 | — | — |
| | Vol. per min., litres | 8·0 | 7·0 | 8·0 | 9·5 | 10·5 | 11·5 | 12·5 | 15·0 | 26·5 | 48·0 | — | — |
| Capt. F. I | Rate per min. | 8 | 9 | 9 | 10 | 10 | 9 | 9 | 9 | 9 | 10 | 12 | 15 |
| | Vol. per resp., c.c. | 885 | 775 | 725 | 735 | 765 | 1015 | 715 | 875 | 685 | 940 | 1135 | 815 |
| | Vol. per min., litres | 7·0 | 7·0 | 6·5 | 7·5 | 7·5 | 9·0 | 6·5 | 8·0 | 6·0 | 9·5 | 13·5 | 12·5 |
| Capt. F. II | Rate per min. | 8 | 8 | 10 | 10 | 8 | 10 | 9 | 8 | 7 | 10 | 11 | — |
| | Vol. per resp., c.c. | 680 | 700 | 660 | 780 | 1080 | 585 | 865 | 1030 | 1010 | 590 | 790 | — |
| | Vol. per min., litres | 5·5 | 5·5 | 6·5 | 8·0 | 8·5 | 6·0 | 8·0 | 8·0 | 7·0 | 6·0 | 9·0 | — |
| Capt. McC. | Rate per min. | 11 | 9 | 9 | 9 | 7·5 | 8 | 12 | — | — | — | — | — |
| | Vol. per resp., c.c. | 960 | 1015 | 1250 | 985 | 1265 | 1510 | 1795 | — | — | — | — | — |
| | Vol. per min., litres | 10·5 | 9·0 | 11·5 | 9·0 | 9·5 | 12·0 | 21·5 | — | — | — | — | — |
| Cpl. M. I | Rate per min. | 12·5 | 13·75 | 13 | 12·5 | 13 | 14 | 13·5 | 13·5 | 15·5 | 15·5 | — | — |
| | Vol. per resp., c.c. | 1065 | 705 | 960 | 920 | 865 | 995 | 1060 | 1205 | 1335 | 1645 | — | — |
| | Vol. per min., litres | 13·5 | 10·5 | 12·5 | 11·5 | 11·0 | 14·0 | 14·5 | 16·5 | 20·5 | 25·5 | — | — |
| Cpl. M. II | Rate per min. | 10 | 10 | 10 | 10·5 | 11 | 10·5 | 13·5 | 16 | 15 | 16 | — | — |
| | Vol. per resp., c.c. | 1110 | 1025 | 870 | 1105 | 730 | 900 | 770 | 795 | 925 | 995 | — | — |
| | Vol. per min., litres | 11·0 | 10·0 | 8·5 | 11·5 | 8·0 | 9·5 | 10·5 | 12·5 | 14·0 | 16·0 | — | — |
| J. S. H. I | Rate per min. | 14 | 15 | 17 | 19·5 | 25·5 | 28 | 32 | 40 | — | — | — | — |
| | Vol. per resp., c.c. | 745 | 860 | 870 | 1020 | 945 | 995 | 990 | 905 | — | — | — | — |
| | Vol. per min., litres | 10·5 | 13·0 | 14·0 | 20·0 | 24·0 | 28·0 | 31·5 | 36·0 | — | — | — | — |
| J. S. H. II | Rate per min. | 15 | 16 | 17 | 21 | 26 | 29 | 36 | — | — | — | — | — |
| | Vol. per resp., c.c. | 680 | 730 | 625 | 795 | 795 | 795 | 890 | — | — | — | — | — |
| | Vol. per min., litres | 10·0 | 11·5 | 10·5 | 16·5 | 20·5 | 23·0 | 32·0 | — | — | — | — | — |
| J. S. H. III | Rate per min. | 11·5 | 12 | 14 | 18·5 | 22 | 25 | 29 | 31 | — | — | — | — |
| | Vol. per resp., c.c. | 1050 | 1405 | 1720 | 1575 | 1510 | 1740 | 1615 | 1780 | — | — | — | — |
| | Vol. per min., litres | 12·0 | 17·0 | 24·0 | 29·0 | 33·5 | 43·5 | 47·0 | 55·0 | — | — | — | — |

this is that the respiration can adapt itself to changes in the proportion of $CO_2$ in the air breathed without overshooting the mark. In the case of oxygen however where the solubility is so much less, changes of tension occur with great rapidity after changes of amount and hence

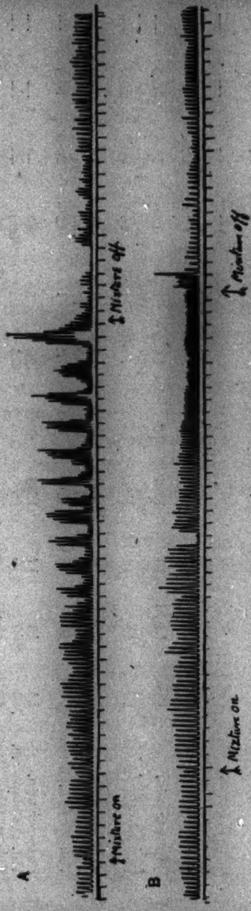


Fig. 4. Subject J. G. P. Time marker = 10 seconds.
(a) Breathing 10·33 p.c. oxygen, (b) Breathing 9·59 p.c. oxygen.

the local effect of want of oxygen on the respiratory centre is very rapid and the consequent result is periodicity of the breathing.

Where the proportion of oxygen is still further reduced this last

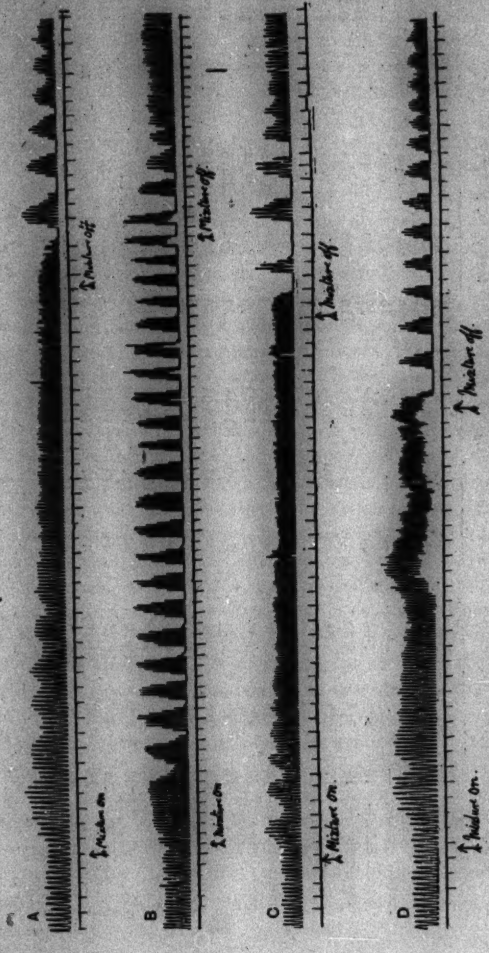


Fig. 5. Subject J. S. H. Time marker = 10 seconds. (a) Breathing 11·19 p.c. oxygen, (b) Breathing 11·05 p.c. oxygen + 0·7 p.c. $CO_2$, (c) Breathing 10·64 p.c. oxygen, (d) Breathing 9·84 p.c. oxygen.

TABLE V.

| | J. S. H. 11·19 % $O_2$ | | | J. S. H. 10·64 % $O_2$ | | | J. S. H. 9·84 % $O_2$ | | | J. G. P. 9·59 % $O_2$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate per resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate per resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate per resp. per min. | Vol. per resp. c.c. | Vol. per min. litres | Rate per resp. per min. | Vol. per resp. c.c. | Vol. per min. litres |
| Prelim. control period breathing air | — | — | — | — | — | — | 15 | 559 | 8·39 | — | — | — |
| | 16 | 496 | 7·94 | 20 | 465 | 9·30 | 17 | 599 | 10·18 | 15 | 420 | 6·30 |
| | 18 | 514 | 9·25 | 21 | 452 | 9·45 | 16 | 568 | 9·09 | 15 | 407 | 6·11 |
| | 17 | 519 | 8·82 | 23 | 443 | 10·18 | 17·5 | 563 | 9·85 | 16 | 438 | 7·01 |
| Period of experiment breathing mixture | 16 | 653 | 10·45 | 23 | 626 | 14·40 | 18 | 877 | 15·79 | 15·5 | 541 | 8·39 |
| | 18 | 839 | 15·09 | 27 | 604 | 16·30 | 20 | 881 | 17·62 | 16 | 644 | 10·30 |
| | 20 | 693 | 13·86 | 29 | 563 | 16·33 | 26 | 863 | 22·44 | 16 | 555 | 8·88 |
| | 22 | 716 | 15·74 | 32 | 590 | 18·88 | 30 | 792 | 23·76 | 18 | 608 | 10·94 |
| | 23 | 648 | 14·91 | 35 | 541 | 18·94 | 36 | 680 | 24·48 | 18 | 689 | 12·40 |
| | 25 | 568 | 14·20 | 38 | 492 | 18·30 | 36 | 783 | 28·19 | 18 | 501 | 9·02 |
| | 26 | 563 | 14·65 | 40 | 510 | 20·40 | 42 | 653 | 27·43 | 20 | 546 | 10·92 |
| | 28 | 548 | 15·35 | 42 | 474 | 19·91 | 50 | 568 | 28·40 | 20 | 470 | 9·40 |
| | 30 | 523 | 15·70 | 44 | 425 | 19·80 | 52 | 528 | 27·46 | 26 | 443 | 11·52 |
| | 34 | 537 | 18·25 | 44 | 443 | 19·49 | — | — | — | 37 | 349 | 12·91 |
| | 35 | 496 | 17·37 | 43 | 447 | 19·22 | — | — | — | 54 | 282 | 15·23 |
| | 36 | 479 | 17·23 | 44 | 443 | 19·49 | — | — | — | 68 | 313 | 21·28 |
| | 38 | 465 | 17·67 | 45 | 465 | 20·93 | — | — | — | — | — | — |

effect is replaced by another. This is seen in the tracings which show that the periodicity of the breathing is succeeded by a very rapid, shallow type of respiration. The serious nature of this result will become apparent after consideration of the paper on the effects of shallow breathing, and it will be sufficient to mention here the main conclusion reached in that paper, which is that shallow breathing in itself is a cause of anoxæmia. Thus a very dangerous vicious circle tends to be set up. Want of oxygen in the inspired air causes shallow breathing, which in turn intensifies the anoxæmia.

In seeking for an explanation of this effect of want of oxygen one must bear in mind (1) the effect of fatigue, which will be discussed in a later paper, (2) the great sensitiveness of the tissues to want of oxygen. The body is unable to store oxygen to any appreciable extent and hence the effects of low oxygen percentage in the inspired air are immediately felt by the tissues and their functional activity is greatly impaired by any interference with the oxygen supply. The grave effects of interrupting the circulation for a short time through such an organ as the kidney are well known, and it appears probable that the quickening and shallowing of the respiration when low oxygen percentages are breathed is due to impairment of the vitality of the respiratory centre, which is unable to perform its function adequately when starved of oxygen.

The above description of the respiratory effects of want of oxygen applies to the results observed on the whole. There are however individual differences. In some subjects the lowering of the threshold for $CO_2$ and consequent hyperpnœa is a more conspicuous result than in others. In such cases the dangerous effects of want of oxygen will tend to be somewhat postponed owing to the increased efficiency of the lung ventilation and consequent efficient use of such oxygen as is present in the air breathed.

This fact would seem to afford an explanation of the much greater liability of some people than others to mountain sickness. Those who react to want of oxygen pre-eminently by shallow breathing will be more seriously affected under conditions of lowered barometric pressure than those whose reaction involves a great alteration of $CO_2$ threshold.

Finally the tracings also show well the onset of periodic breathing when normal air is substituted for the low oxygen mixture breathed. The explanation of this phenomenon is evidently that given by Haldane and Douglas for Cheyne-Stokes breathing after forced breathing.

We wish to acknowledge the assistance we have received from the Medical Research Committee.

## SUMMARY.

1. The respiratory response to anoxæmia is in three stages.

(a) Increased depth of respiration, and increased ventilation per minute owing to lowered $CO_2$ threshold.

(b) Periodic breathing unless the anoxæmia is considerable.

(c) Frequent and correspondingly shallow breathing.

2. Excess of $CO_2$ (increased hydrogen ion concentration) causes a considerable and persistent increase in depth of respiration and relatively slight increase in frequency. This response is in marked contrast to the response to want of oxygen.

3. The maximum increase in lung ventilation is obtained when excess of $CO_2$ and anoxæmia are both present.

# THE EFFECTS OF SHALLOW BREATHING.

By J. S. HALDANE, M.D., F.R.S., J. C. MEAKINS, M.D., Lt-Col., C.A.M.C. and J. G. PRIESTLEY, M.C., B.M., Capt., R.A.M.C.

*(From the Clinical Laboratory, No. 15 Canadian General (Duchess of Connaught's Red Cross) Hospital.)*

The question as to the means by which the respiration is regulated so as to harmonise with the metabolism of the body has been the subject of many investigations. These have in the main led to two theories as to the nature of this regulation, namely (*a*) that it is a matter of reflex nervous control, and (*b*) that it is a chemical regulation.

Following on the classical research of Hering and Breuer (1) in 1868 the first theory became predominant, but a paper published by Haldane and Priestley (2) in 1905 led to a recognition of the fundamental predominance of the chemical regulation. A period then followed in which the nervous mechanism of the regulation of respiration did not receive so much attention and was indeed largely neglected until Haldane and Mavrogordato (3) investigated the relation between the chemical regulation and the Hering-Breuer reflex. Their results clearly demonstrated for the first time the connection between the nervous control and the chemical control.

Recent investigations carried out in this laboratory into the cause of the symptoms met with in men suffering chronically from the effects of gas poisoning, and from a group of symptoms known as D.A.H., "Soldier's Heart," "Irritable Heart," "Effort Syndrome," etc., have turned our attention to the importance of the regulation of the depth of respiration and have led to results which are of much physiological importance. A summary of these investigations in their more practical aspects was given in a report circulated for official purposes[1]. The present paper is a full account of one part of that work, namely the experimental investigation of the effects of shallow breathing.

[1] *Reports of the Chemical Warfare Medical Committee.* No. 5. "The Reflex restriction of respiration after gas poisoning."

The reflex first described by Hering and Breuer has been very widely misunderstood, and indeed as described in the physiological textbooks of recent years is made to appear as a reflex singularly perfectly adapted to prevent the maintenance of efficient respiration. Reference to Hering and Breuer's original paper and the tracings therein contained shows clearly that the inspiratory and expiratory impulses from the centre are sent out in response to afferent impulses passing up the vagus and initiated by the state of collapse or inflation of the lung. Hering and Breuer therefore correctly speak of an automatic regulation of the respiratory movements, but their experiments afford no explanation of the means by which the breathing is kept slow and deep at one time and fast and shallow at another. There was indeed no clear understanding of how the regulation of the breathing by the Hering-Breuer reflex can be reconciled with the obvious fact that the breathing is also normally so regulated as to be deep or shallow according to the needs of the body until the question was investigated by Haldane and Mavrogordato(3). Soon after Hering and Breuer's discovery the question of this reflex was taken up by Head(4) who worked in Hering's laboratory and who employed for the first time a new method of obtaining respiratory records, namely, recording the contractions of a slip of a rabbit's diaphragm. Unfortunately this method resulted in focussing attention exclusively on the inspiratory part of the respiratory acts, so that Head's interpretation of the nervous control of breathing leaves out of account the expiratory side of the reflex and thus gives an entirely erroneous impression.

Owing to this paper and to the misleading accounts of the work of Hering and Breuer which became current, understanding of the meaning and importance of this reflex became obscured and when Haldane and Priestley showed the importance of the part played by $CO_2$ in regulating the ventilation of the lungs, interest in the nervous control of the depth of respiration became lessened. It also happened that the experiments of Haldane and Priestley seemed to indicate that it was a matter of no great importance whether the lung ventilation was effected—within limits—by slow and deep or by rapid and shallow respirations.

Later, when Haldane and Mavrogordato again investigated the Hering-Breuer reflex and showed that it is controlled by the $CO_2$ tension of the blood they still regarded the depth of respiration as a matter of secondary importance, and indeed some experiments performed by Haldane seemed to show that the ventilation of the lungs could be equally well maintained at respiratory rates varying from 2 to 60. Haldane(5) found that if the rate of respiration was voluntarily maintained at either of these figures—allowing the depth to adjust itself—the alveolar $CO_2$ remained constant; but it must be noted that these experiments were of very short duration—about 2 minutes—and that it is practically certain that periodicity in the depth of breathing occurred but escaped notice.

Haldane and Priestley(2) had also found that alveolar $CO_2$ remained constant while the rate of respiration was voluntarily varied from 9 to 30 per minute, the depth being allowed to adjust itself. When, however, we came to investigate the respiratory phenomena of soldiers suffering from chronic effects of gas poisoning and "Irritable heart" we were forced to reconsider the interpretation of these experiments.

As is well known the condition known as "Irritable heart" is characterised by breathlessness on exertion, rapid pulse, fainting attacks, giddiness, exhaustion, lassitude, headache, irritability, etc. We found that the patients subject to this condition show invariably a remarkable type of breathing. The respiration is rapid—from 20 to 60 or more per minute—and shallow—250 to 350 c.c. or thereabouts—and on exertion the rate increases very abnormally while the increase in depth is abnormally small. It was also found that their alveolar $CO_2$ is abnormally low[1]. Hunt and Price Jones[2] drew attention to the occurrence of polycythæmia in chronic cases and Barcroft[3] described a condition of "acidosis" in these patients. All these conditions are such as would arise if the patient were short of oxygen either intermittently or continuously, and this conclusion is confirmed by the very definite result obtained when they breathe air enriched with oxygen. In fact the patients are in the same state as a normal individual at high altitudes where the diminished oxygen tension of the inspired air produces the same series of effects. We found that when they are breathing oxygen the patients are able to do an amount of muscular work which is quite beyond their powers when they breathe air only, and they state emphatically that the oxygen relieves their subjective symptoms. In many of the patients exhibiting this condition there is no sign of any serious structural change in heart and lungs. All these patients however show the shallow rapid type of breathing.

---

[1] C. G. Douglas' report forwarded to G.H.Q. France. Drury. Medical Research Committee Report upon Soldiers returned as cases of "Disordered action of the heart," p. 19. 1917.

[2] Report forwarded to G.H.Q. France.

[3] Barcroft. C.W.M.C. report.

Since, then, some of their symptoms were clearly due to want of oxygen and since a constant abnormality found was the peculiar type of breathing, the question arose as to whether this type of respiration could be the cause of the oxygen-want, although the experiments of Haldane mentioned above had apparently indicated that this could not be the case.

In order to investigate this matter we determined to imitate the condition of shallow breathing by restricting the volume of air per breath inhaled by normal individuals.

The apparatus used consisted of a collapsible bellows of india-rubber of suitable size. Two such "concertinas" were used in different experiments—one had a capacity when distended of 3½ litres; the other of ½ litre. The top and bottom of the concertina were formed by rigid pieces of wood, the top being suspended in a fixed position on a suitable stand. To the middle of the bottom plate was fixed a metal rod which passed through a metal tube and acted as a guide, thus ensuring that the only movement of the bottom of the concertina was a vertical up and down movement. Surrounding the tube through which the guide rod passed was another tube which could be clamped at any height desired by means of a screw, so that the downward excursion of the bottom of the concertina was limited. Fixed to the bottom plate of the concertina was a writing pen which recorded its movements. The whole was mounted on a stand provided with levelling screws; and the three uprights by means of which the concertina was suspended were threaded at the top and provided with nuts so that the large and small concertinas could be readily exchanged and the top in each case levelled as required. Through the top plate of the concertina passed a short metal tube, of 1 inch internal diameter and bent to a right angle. This tube connected the concertina to the remainder of the apparatus which is now to be described. (See Fig. 1.)

To a mouthpiece was attached a tube of 1 inch diameter provided with inspiratory and expiratory valves; the whole being so proportioned as to reduce the dead space as much as possible. From the part of this tube opposite the mouthpiece and between the inspiratory and expiratory valves a small tube ¼ inch in diameter led to a delicate tambour which thus became distended on expiration and collapsed on inspiration. This tambour was made to control a make and break mercury contact in an electrical circuit which included a powerful electro-magnet the use of which is described below. This arrangement was described by Yandell Henderson(6). The lower end of the tube carrying the mouthpiece below the inspiratory valve was connected by means of a piece of rubber tubing as short as conveniently possible to another short piece of 1 inch metal tubing bent at a right angle and supported on a wooden stand. The upright part of this tube contained a mica valve which opened on inspiration, and on the upper side of the horizontal part was the seating of another mica valve which closed on inspiration. To this valve was firmly attached an iron armature situated just below the electro-magnet mentioned above. The open end of the horizontal part of the tube carrying the valves was attached to the tube leading through the top of the concertina.

The effect of the arrangement described above is as follows: during inspiration air is drawn from the concertina through the inspiratory valves to the lungs. At the same time the slight negative pressure is enough to cause the tambour to collapse and so break the circuit of the electro-magnet. Consequently the armature attached to the valve on the horizontal tube is not attracted and this valve remains closed. The only air therefore which can be inspired is that contained in the concertina and its amount is determined by the height at which the stop supporting the bottom of the concertina is set. On expiration the expired air escapes through the expiratory valve of the mouthpiece, and at the same time the inspiratory valves close. Simultaneously the slight expiratory pressure distends the tambour, which thus closes the circuit of the electro-magnet. The valve below the magnet is thus drawn up and thus a clear way is made from the outside air to the interior of the concertina. The bottom of the concertina falls by its own weight to the stop and thus a definite quantity of fresh air is drawn in ready for the next inspiration. The same sequence of events can then be repeated indefinitely. It should be clear therefore that the effect of the apparatus is to provide a constant and limited quantity of fresh air for each inspiration and at the same time give a quantitative record of the respiration, for the concertina may easily be calibrated. //

A further arrangement consists of a wide glass tube which is fixed round the electro-magnet and the valve controlled by it. The ends of this glass tube are closed by rubber bungs to make airtight joints. Through the upper of these rubber bungs a smaller glass tube about ¼ inch in diameter passes. This arrangement makes it easy to supply fresh air, oxygen or any desired gaseous mixture of known composition to the concertina. The annexed Fig. 1 shows the apparatus. The apparatus can of course also be used to obtain a record of the normal breathing. All that is necessary is to use a concertina of greater capacity than the volume of the respirations. The record is of course of the inspirations only. The expirations are not recorded.

**Miller** has published several very interesting papers in which he has worked out the structure of the lungs(7). He has shown that each terminal bronchiole ends in a cavity which he terms an atrium. Out of the atrium open several air sacs. The walls of both atrium and air cells are provided with alveoli. The atria act in effect as distributing chambers and ensure an even division of the inspired air to alveoli (containing by far the greater portion of the air in the lungs) which lie beyond the atria. Were it not for this arrangement the air cells near the termination of the bronchiole would receive a greater amount of the entering air in proportion to their nearness, and consequently the ventilation of the air sacs would be very uneven, and the fact that the deeper alveolar air is uniform in composition, as **Haldane** and **Priestley** found, would be unintelligible.

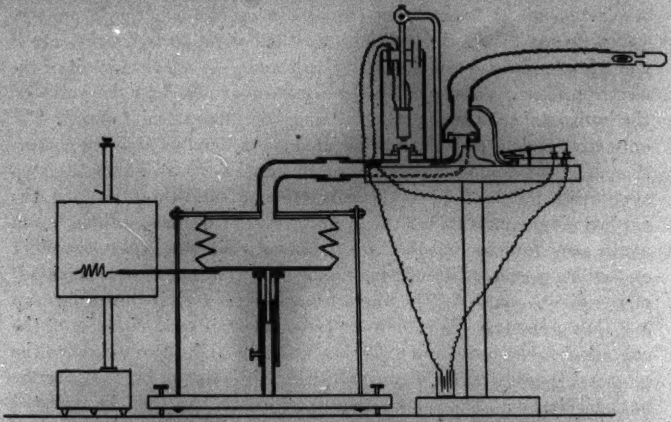
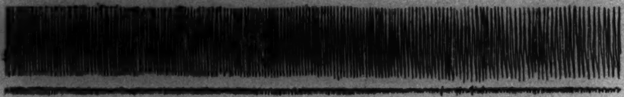


Fig. 1. "Concertina" apparatus for continuous record of respiration.

At first we expected that by suitably adjusting the concertina so as to limit the volume of air inspired we might be able to so restrict the air entry as to cause the ventilation not to extend appreciably beyond the atria, leaving the air sacs full of stagnant or nearly stagnant air. If this were the case we might be able to detect a considerable difference between the respiratory quotients of the expired air and the deep alveolar air: for it is clear from the shapes of the dissociation curves of blood for $CO_2$ and $O_2$ that there is practically no limit to the extent to which over-ventilation can remove $CO_2$ from the blood circulating through the well ventilated atria on the one hand, while on the other the amount of oxygen given up to this blood cannot be increased to any appreciable extent by increasing the ventilation beyond the normal. The respiratory quotient in the atria should therefore be high if the hypothesis as to relatively excessive ventilation of the atria were correct. In the air-sacs, however, where the air is supposed to be stagnant, oxygen will continue to be taken up by the blood after equilibrium is established between blood and air as regards $CO_2$. The respiratory quotient of the air-sac air or deep alveolar air should thus fall.

When we came to perform the experiment however this expectation was not borne out. With the concertina so adjusted that the volume of air taken in at each inspiration was only slightly below the normal the rate of respiration quickened correspondingly but there was no other apparent change. The lung ventilation could it seemed, be carried on indefinitely with the new rate and volume of respiration. There was no definite and constant difference between the respiratory quotients of the expired air and the deep alveolar air. Fig. 2 gives a record obtained under these conditions and Table I gives the respiratory quotients of expired air and deep alveolar air at various rates and depths of inspiration.



All curves are read from left to right. Inspiration being the upstroke.

Fig. 2. Subject J. C. M. Time marker = seconds.

TABLE I. Subject J. G. P.

| Vol. per resp. (c.c.) | Resp. per min. | Vol. per min. (ltrs) | Expired air | | | Deep alveolar air | | | Bar. |
|---|---|---|---|---|---|---|---|---|---|
| | | | CO % | O % | R. Q. | CO % | O % | R. Q. | |
| 161 | 106 | 17·1 | 2·01 | 18·44 | ·76 | 5·71 | 14·06 | ·79 | 758 |
| 155 | 106 | 16·4 | 2·10 | 18·35 | ·76 | 6·22 | 12·85 | ·72 | 758 |
| 147 | 69 | 10·1 | 2·97 | 17·38 | ·78 | 5·63 | 13·55 | ·71 | 758 |
| 105 | 118 | 12·3 | 2·36 | 17·90 | ·71 | 5·42 | 14·45 | ·79 | 760 |

The concertina was then closed down further but no particular difference in the result was noticed till the inspired air was so much limited in amount that the rate of respiration went up to about 100 to

150 per minute. Under these circumstances the experiment was at first rather difficult to perform because the apparatus only fixed the amount of air that could be inspired, but constant attention and exercise of the will was required to overcome the natural tendency of the Hering-Breuer reflex and make the volume of expiration correspond to the volume of inspiration. However this became comparatively easy with a little practice, and one could prevent the lungs becoming more and more inflated or deflated during an experiment. The result was continually increasing discomfort and sense of suffocation until the experiment had to be stopped. The respiratory quotient of the deep alveolar air fell to 0·5 and that of the expired air also fell.

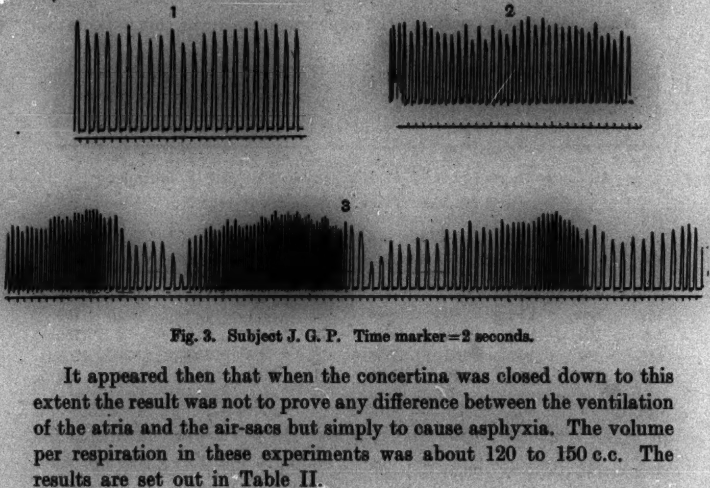


Fig. 3. Subject J. G. P. Time marker = 2 seconds.

It appeared then that when the concertina was closed down to this extent the result was not to prove any difference between the ventilation of the atria and the air-sacs but simply to cause asphyxia. The volume per respiration in these experiments was about 120 to 150 c.c. The results are set out in Table II.

TABLE II.

| | | | | Expired air | | | Alveolar air | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Vol. per resp. (c.c.) | Resp. per min. | Vol. per min. (ltrs) | $CO_2$ % | $O_2$ % | R. Q. | $CO_2$ % | $O_2$ % | R. Q. | Bar. |
| J. G. P. | — | 108 | — | — | — | — | 7·23 | 9·02 | ·54 | — |
| | 64 | 116 | 7·47 | 1·03 | 19·21 | ·50 | 6·83 | 8·03 | ·47 | — |
| | 141 | 102 | 14·47 | 2·03 | 18·29 | ·71 | 6·38 | 7·77 | ·64 | 758 |
| | 159† | 156 | 24·9 | 2·32 | 17·73 | ·67 | 7·40 | 10·76 | ·67 | 755 |
| J. C. M. | 142 | 116 | 16·44 | 1·72 | 18·66 | ·69 | 6·61 | 12·62 | ·75 | 764 |
| | 115 | 80 | 9·2 | 1·02 | 19·45 | ·64 | 6·79 | 8·56 | ·48 | — |

† Doing light work. Cyanosis and periodic breathing on stopping.

On stopping the concertina breathing, however, it was noticed that there was a slight tendency to periodic breathing. This was an indication that the effect of the restricted breathing was something more than simple asphyxia.

We therefore began a new series of experiments in which the concertina was first adjusted so as to allow a full normal inspiration and was later closed down very gradually step by step. The breathing from the concertina at each stage was continued for a considerable time. As before there was at first no change beyond increase in rate of respiration corresponding to the restricted volume. But when the restriction in volume was carried to a very slight degree beyond a certain point a new phenomenon appeared. After breathing for some time with the concertina thus adjusted periodicity in the respiration began to appear. At first slight variations in the rate of respiration became noticeable, but with proper adjustment of the concertina the periodicity became accentuated and periods of complete apnœa alternated with periods of extremely rapid and urgent respiration. Owing of course to the restriction in volume caused by the concertina the periodicity manifested itself rather by changes in rate than by changes in volume. Fig. 3 gives a record showing this effect.

The clinical type of periodic respiration, *i.e.* Cheyne-Stokes breathing, was investigated by Pembrey and Allen (8), who showed that the periodicity was abolished and regular breathing restored by adding oxygen or $CO_2$ to the air inspired or by diminishing the oxygen of the inspired air. They attributed the periodicity to diminished excitability of the nervous system. Later the subject was further investigated by Douglas and Haldane (9) who proved that periodic breathing is unmistakeable evidence of anoxæmia. Confirmation of the conclusion that the periodic breathing produced by our concertina experiments was due to anoxæmia was at once obtained on adding a small proportion of oxygen to the air entering the concertina. The effect of this was to abolish completely the periodicity and restore regular but rapid breathing. Fig. 4 reproduces a record showing this result.

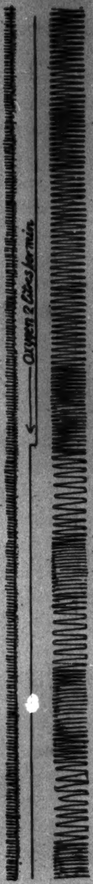


Fig. 4. Subject J. G. P. Time marker = seconds.

The periodic breathing produced by restriction of the volume of air entering the lungs could be continued more or less indefinitely without discomfort, the periods gradually lengthening as $CO_2$ was washed out of the blood.

TABLE III.

| Subject | Vol. per resp. (c.c.) | Resp. per min. | Vol. per min. (ltrs) | Expired air CO % | Expired air O % | Expired air R. Q. | Alveolar air CO % | Alveolar air O % | Alveolar air R. Q. | Bar. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| J. G. P. | 192 | 40 | 7·67 | 2·82 | 17·9 | ·9 | — | — | — | — | Mixed hypopnœa and hyperpnœa |
| | 190 | 26 | 4·90 | 2·98 | 17·53 | ·86 | 5·05 | 14·66 | 0·76 | — | Hypopnœic period |
| | 218 | 60 | 13·15 | 2·67 | 18·38 | 1·04 | 4·63 | 16·36 | 1·00 | — | Hyperpnœic period |
| | 242 | 25 | 6·30 | 2·35 | 17·82 | ·7 | — | — | — | 766 | Hypopnœa |
| | 225 | 60 | 13·50 | 2·53 | 18·10 | ·86 | 4·4 | 16·20 | ·92 | 766 | Hyperpnœa |
| | 250 | 64 | 16·00 | 2·71 | 17·82 | ·83 | 4·55 | 16·62 | 1·06 | 766 | „ |
| | 220 | 20 | 4·38 | 2·56 | 17·28 | ·64 | 5·95 | 11·54 | ·57 | 766 | Hypopnœa |
| J. C. M. | — | — | — | — | — | — | 6·6 | 9·82 | ·53 | 759 | „ |
| | — | — | — | — | — | — | 5·29 | 15·50 | ·96 | 759 | Hyperpnœa |
| | — | — | — | — | — | — | 6·38 | 11·40 | ·61 | 759 | Hypopnœa |
| | — | — | — | — | — | — | 6·00 | 13·08 | ·71 | 759 | „ |
| | — | — | — | — | — | — | 4·88 | 16·11 | 1·00 | 759 | Hyperpnœa |

TABLE IV. (Subject J. G. P.)

| Duration in mins. | Vol. per resp. (c.c.) | Resp. per min. | Vol. per min. (ltrs) | Expired air $CO_2$ % | Expired air $O_2$ % | Expired air R. Q. | Alveolar air $CO_2$ % | Alveolar air $O_2$ % | Alveolar air R. Q. | Bar. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ¼ | — | 60 | — | 2·0 | 18·26 | ·68 | — | — | — | 756 | — |
| 5 | 173 | 68 | 11·78 | 2·27 | 18·75 | ·93 | — | — | — | 756 | — |
| 27 | 212 | 54 | 11·43 | 2·12 | 18·54 | ·84 | — | — | — | 756 | — |
| 38 | — | — | — | — | — | — | 4·83 | 16·23 | 1·02 | 756 | — |
| 60 | 192 | 40 | 7·67 | 2·82 | 17·90 | ·9 | — | — | — | 756 | Breathing periodic |
| 103 | 190 | 26 | 4·9 | 2·98 | 17·53 | ·86 | — | — | — | 756 | Hypopnœic period |
| 108 | 218 | 60 | 13·15 | 2·67 | 18·38 | 1·04 | — | — | — | 756 | Hyperpnœic period |
| 144 | — | — | — | — | — | — | 5·05 | 14·66 | ·76 | 756 | Hypopnœic period |
| 148 | — | — | — | — | — | — | 4·63 | 16·36 | 1·00 | 756 | Hyperpnœic period |

The expired air and alveolar air were analysed both in hypopnœic and hyperpnœic periods and the results are given in Table III.

In Table IV are given the figures from an experiment in which the restricted breathing was continued for a longer time. The table shows the gradual onset of periodic breathing and also shows that the oxygen percentage of the alveolar air was abnormally high just prior to the beginning of the periodicity, and also, at a later stage, during both hypopnœic and hyperpnœic periods.

How then are we to explain the undoubted fact that the subject of the experiment was suffering from anoxæmia at a time when his alveolar air contained a higher percentage of oxygen than normal?

**Haldane** and **Priestley** in the paper referred to above showed that in any individual at rest the alveolar $CO_2$ and $O_2$ are remarkably constant and that even a slight change in the alveolar $CO_2$ has a very great effect on the breathing, a rise of 0·2 p.c. in the alveolar $CO_2$ doubling the ventilation of the lungs. They assumed that this constancy extends to the alveolar air of all parts of the lung. Their experiments however did not exclude the possibility that the "alveolar air" as obtained and analysed by them was an average sample of alveolar air of different composition in the different parts of the lungs.

Now in a very interesting paper on the mechanism of respiration in man Prof. A. **Keith**(10) states that:

(1) The lung is composed of elements of varying degrees of extensibility. Hence the expansion of its parts is unequal during inspiration. The lung may be divided into three zones of different extensibility—(*a*) the root zone, (*b*) the intermediate zone, and (*c*) the subpleural zone.

(2) The lungs do not expand equally in all directions but execute a certain definite movement during inspiration. The expansion of the lung is a regulated act resembling the opening of a Japanese fan rather than the distension of a bladder when the external pressure is reduced. It does not take place instantaneously and equally throughout and consequently part of the lung may be in a state of partial or almost complete disuse.

If then the circulation of the blood is fairly uniform both through the well ventilated and badly ventilated parts of the lungs the result must be that the arterial blood going to the heart must be a mixture of blood one part of which has been exposed to fresh air in greater degree than the other part. Now **Christiansen, Douglas** and **Haldane**(11) have worked out the dissociation curve of blood for $CO_2$ and the effect thereon of varying degrees of saturation of the hæmoglobin with oxygen. Their results show that for the conditions existing in the lungs the curve of dissociation of blood for $CO_2$ approximates for a long distance to a straight line rising somewhat steeply. Fig. 5 gives the dissociation curves of blood for $O_2$ and $CO_2$. Reference to Fig. 5 shows that for the tensions of $O_2$ and $CO_2$ which govern the gaseous exchange between blood and air in the lungs the $O_2$ curve and the $CO_2$ curve are entirely different and that diminished respiratory removal of $CO_2$ from one part of the blood in one region of the lung may be compensated for by increased removal of $CO_2$ from another part of the blood in another region of the lung. Thus it follows that despite uneven ventilation of the lungs the average $CO_2$ tension of the arterial blood is maintained at its normal level. Also just as the $CO_2$ tension of the arterial blood reaching the respiratory centre is the average of the $CO_2$ tensions of blood from well and badly ventilated parts of the lungs, so also is the $CO_2$ in the "alveolar air" an average from alveolar air of higher and lower $CO_2$ content in different parts of the lungs. The one average is correlated with the other and thus it is that so long as the average alveolar $CO_2$ remains at its normal level the ventilation of the lungs, as governed by the activity of the respiratory centre, also remains, *ceteris paribus*, at its normal amount.

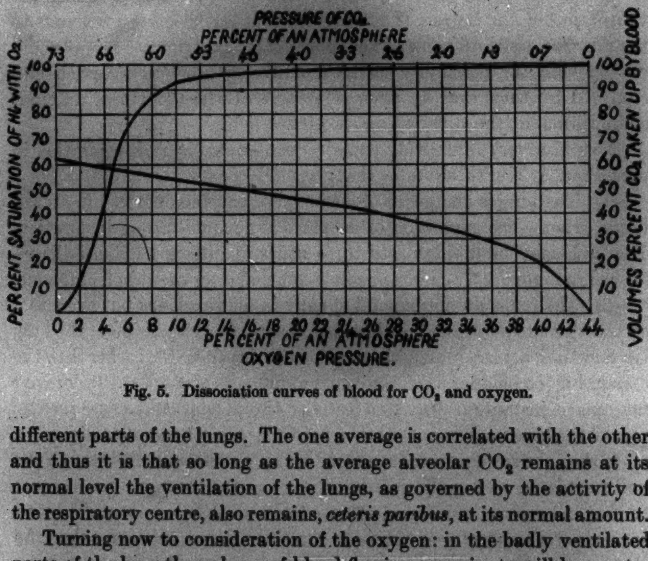


Fig. 5. Dissociation curves of blood for $CO_2$ and oxygen.

Turning now to consideration of the oxygen: in the badly ventilated parts of the lung the volume of blood flowing per minute will be greater in relation to the amount of fresh air entering these parts than in the well ventilated regions. Hence the blood will take up more of the oxygen and the percentage of oxygen in the relatively stagnant alveolar air of the badly ventilated parts will fall below the average. But the figure shows that the dissociation curve of blood for $O_2$ is totally different from the $CO_2$ curve, and that as the oxygen in the stagnant air falls the percentage saturation of the blood passing through these alveoli must also fall greatly. But for oxygen pressures above about 88 mm. Hg. the curve is very flat. Hence any increase of ventilation beyond the amount sufficient to keep the alveolar $CO_2$ at this level is quite unable to add appreciably to the amount of oxygen taken up by the blood. Thus it follows that in the well ventilated parts of the lungs firstly the increased ventilation is incapable of yielding an excess of oxygen to the blood sufficient to compensate for the lack of oxygen in the blood from the badly ventilated parts and secondly, since more than the average amount of fresh air is passing through these alveoli while no more than the normal amount of oxygen is being absorbed by the blood, the air expired from these well ventilated alveoli must be abnormally rich in oxygen. Thus the average composition of the whole alveolar air may remain constant as regards percentage of oxygen, as is normally the case, or, when the breathing is abnormally shallow, the oxygen content of the average alveolar air may rise above the normal level even though at the same time the arterial blood is inadequately supplied with oxygen.

This, then, is the explanation of the figures obtained on analysing the alveolar air during restricted breathing from the concertina as described above; and similar effects must ensue in the case of "Irritable heart" patients who suffer from shallow breathing. If the shallow breathing from the concertina is prolonged a complicating factor comes into play. As shown above the restricted breathing produces some degree of anoxæmia and it is well known that one result of anoxæmia is lowering of the threshold for $CO_2$ of the respiratory centre. Hence, when the restricted breathing is prolonged, the alveolar $CO_2$ gradually falls on the average in the same manner as when the anoxæmia is consequent on lowered barometric pressure. Also if the total ventilation per minute is measured it is found that it is increased by the shallow breathing. This is an obvious consequence of both the relative increase of the dead space and the lowered threshold for $CO_2$.

The discovery that the composition of the air in individual alveoli may differ considerably from that of the mixed alveolar air throws an important new light on various phenomena connected with respiration and the blood gases. Thus it has been found by Fredericq and by Krogh that in experiments with the aerotonometer the oxygen tension of the arterial blood during rest is always lower than in alveolar air, whereas Douglas and Haldane, working with the carbon monoxide method, found the oxygen tension in the arterial blood the same as in the alveolar air, as ought to be the case if the oxygen is passing inwards by simple diffusion. The difference between the results by the two methods was attributed by Douglas and Haldane to the arterial blood having, between the lungs and the carotid artery, lost a slight amount of free oxygen owing to the presence of "reducing substances." This explanation is now quite unnecessary. Evidently the carbon monoxide method gives the average of the oxygen tension in the blood leaving individual alveoli, while the aerotonometer method gives the oxygen tension of mixed arterial blood; and the two values are, as explained above, not the same. From the want of agreement between the two values it also seems to follow that even during normal breathing the ventilation of different parts of the lungs is not even. The slightly increased proportion of oxygen found in the arterial blood of animals during muscular exertion or forced artificial respiration is probably due to more even ventilation of the lungs.

It is evident also that at diminished atmospheric pressures the effects of uneven ventilation will, other things being equal, be exaggerated. In this way we can understand the fact that, as was very clearly shown by Miss Fitzgerald, even a comparatively small diminution of atmospheric pressure has a marked influence on the hæmoglobin percentage and alveolar $CO_2$ pressure in persons living under the diminished pressure.

The observation that the shallow breathing patients referred to above suffer from intensified symptoms at night led us to consider the question of the effect of posture on the respiration.

In order to obtain quantitative records of the respiration in the recumbent as contrasted with the upright posture we arranged an apparatus in which the breathing circuit comprised a mouthpiece with inspiratory and expiratory valves which were connected to the two ends of a galvanised iron cylinder of about 50 litres capacity. The end of this cylinder remote from the mouthpiece was also connected by a T-piece and rubber tube to the concertina or other recording apparatus. If the concertina was used it was connected direct to the cylinder, the valves and electromagnet, etc., not being used. Thus true records of expiration were obtained as well as records of inspiration. A soda-lime purifier could also be introduced into the circuit if desired, or the cylinder could be filled with oxygen or other gaseous mixture in place of air. The size of the cylinder (50 litres) allowed of a fair length of record of the respiration being obtained before the effect of accumulating $CO_2$ or diminishing oxygen became noticeable. The whole apparatus, except the recording part, was slung from the ceiling so that the mouthpiece could be comfortably adjusted either in the lying or sitting position. Of course if it was not desired to obtain records of expiration as well as inspiration the complete concertina apparatus including valves and magnet could be used in place of the cylinder and a continuous record obtained.

The following tracings show the records both sitting and lying.

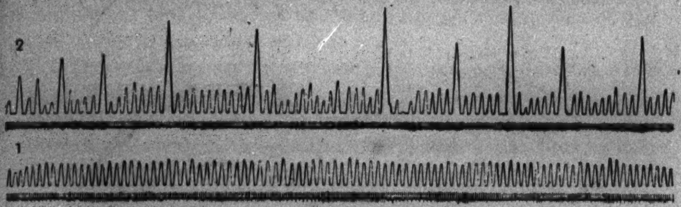


**Fig. 6.** Subject J. C. M. Unlimited concertina. Time in seconds.
1. Sitting. 2. Lying.

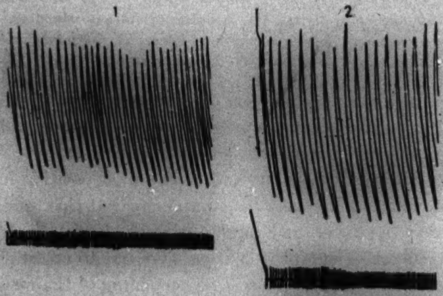


**Fig. 7.** Subject J. S. H. Rebreathing in and out of 50 litre cylinder. Time marker = 2 seconds. 1. Sitting. 2. Lying.

The tracings show, in accord with many other experiments, not quoted, that on lying down the respiration becomes slower and deeper, but they also show that a remarkable type of periodic breathing becomes evident in the case of some subjects when the recumbent position is assumed. In other subjects the breathing becomes slower and deeper but remains perfectly regular.

Thus it seems that the effect of lying down is to accentuate the irregularity in the expansion of the lungs which was referred to above and thus cause a definite though slight anoxæmia even in normal individuals. The probability that this is the case is increased when we consider the annexed tracings from X-ray photographs.

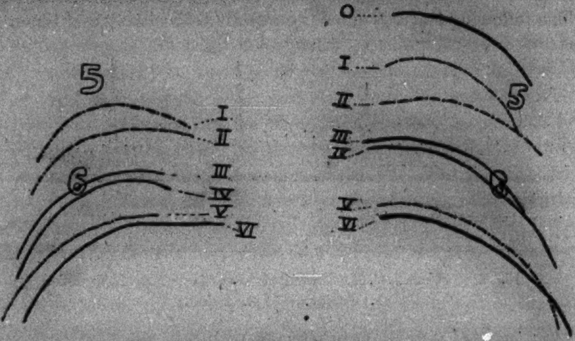


Fig. 8. 0, Deep expiration—standing. I, Deep expiration—lying. II, Moderate inspiration and moderate expiration—lying. III, Moderate expiration—standing. IV, Moderate inspiration—standing. V, Deep inspiration—lying. VI, Deep inspiration—standing. 5 and 6 are marks fixed on 5th and 6th costal cartilages.

These tracings show the position of the diaphragm in full inspiration, normal expiration and deep expiration both standing up and lying down. The photographs were taken from a normal individual.

In connection with the effect of the recumbent position in producing a tendency to anoxæmia it may be pointed out that this tendency would be still more pronounced and might even be definitely deleterious if another effect of the recumbent posture were not to cause deepening of the breathing as described above. This deepening of the respiration will obviously tend to diminish the inequality of the ventilation of different parts of the lungs and will also minimise the anoxæmia which results therefrom. Further evidence that the recumbent position tends to increase the irregularity of the lung expansion was obtained by means of the concertina. This was adjusted so as to produce periodic breathing when the subject was sitting. It was then gradually opened out until the periodicity definitely disappeared. The subject of the experiment then lay down and again breathed from the concertina adjusted at that depth which was found just not to cause periodic breathing in the sitting position. The result always was that the breathing became periodic again as shown by the tracings in Fig. 9.

We have here then further proof that there is a tendency towards the production of anoxæmia when the recumbent position is assumed. In normal individuals this is of no great moment, for the development of a harmful degree of anoxæmia is prevented by the protective effect of the deepening of the respiration which also occurs as a consequence of lying down, and in addition the periodical deep breaths which occur in some persons tend to correct the lack of oxygen should there be any slight degree of anoxæmia.

In those cases however where there is some other cause at work which interferes with the proper oxygenation of the blood the matter is quite otherwise. For instance we may consider the conditions existing in a case of heart disease with impairment of the circulation. Here we have

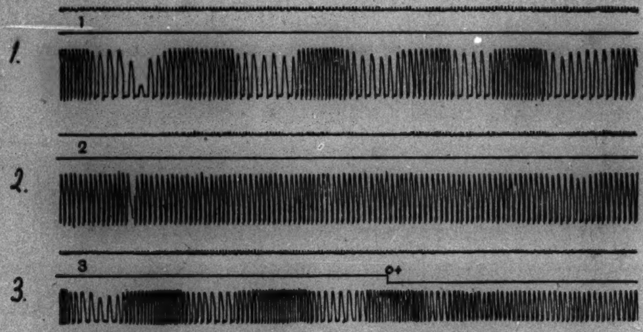


Fig. 9. Subject J. G. P. 1, Breathing restricted by concertina—lying. 2, Breathing restricted to same extent—sitting. 3, Breathing further restricted—sitting. Oxygen given. Curves read left to right. Inspiration upstroke. Time marker = seconds.

a state of affairs in which there is an abnormally great drain on the oxygen carried by the arterial blood, and consequently there is a condition of anoxæmia in the systemic capillaries. Now experiments, which were published in a previous paper, have shown that in the majority of individuals lack of oxygen acts upon the respiratory centre in a way which is strongly contrasted with the effect of excess of $CO_2$. Excess of $CO_2$ causes in the main deepening of the respiration; lack of oxygen causes in the main shallow and quick breathing. Hence in such a case as that under consideration the deficiency of oxygen due to the impairment of the circulation will tend to cause shallowness of the breathing; and this as we have seen prevents the proper oxygenation of the blood in the lungs. It is true that the ineffectiveness of the circulation will also tend towards accumulation of $CO_2$ in the blood; but any excess, as we have seen, can be disposed of by the lungs whether the breathing be shallow or deep. We may suppose that there are patients in whom the circulation is so slightly impaired that as long as the blood is efficiently oxygenated in the lungs there is no great harm done. Equally it is to be expected that there are other patients in whom the ineffectiveness of the circulation reaches such a degree as just to make the respiratory centre begin to respond to anoxæmia, *i.e.* in whom the breathing tends to become shallow. In both cases the patient may be able to carry on fairly comfortably as long as he remains at rest in an upright position. But in the latter case as soon as he lies down his condition alters for the worse. He still has the abnormally great drain of oxygen in the systemic blood and in addition the oxygenation of the blood in the lungs is no longer so

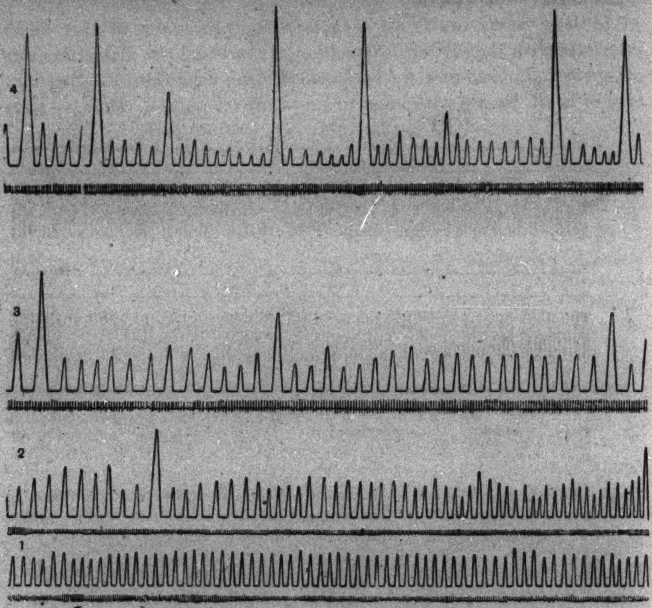


Fig. 10. Subject J. G. P. Concertina unlimited. Time marker = seconds.
1. Sitting. 2. Lying. 3. Sitting, with corsets. 4. Lying, with corsets.

efficiently carried out and consequently the arterial blood carries a less quantity of oxygen than before. There is therefore a condition in which an increased call on the oxygen carried by the blood is coincident with a diminished saturation of the hæmoglobin in the arterial blood with oxygen. Naturally therefore it is to be expected that there will be an exacerbation of symptoms when the patient lies down.

We have now a rational explanation of the phenomenon of orthopnœa, which has hitherto been a complete mystery; and, further the facts detailed above afford a clear indication as to the means to be adopted for the symptomatic treatment of this condition, which is a matter of considerable importance to the patient. Since the orthopnœa is due to shallow breathing caused by and intensifying, anoxæmia, it is clear that the condition can be treated by adding oxygen to the air inspired.

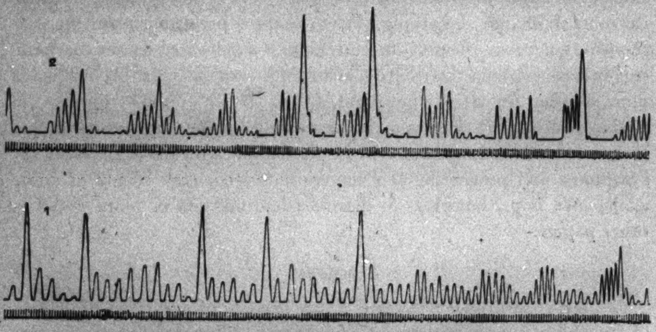


Fig. 11. Subject J. C. M. Unlimited concertina. Time marker = seconds.
1. Sitting, with corsets. 2. Lying, with corsets.

Having found that the uneven ventilation of the lungs had such striking consequences we were led to investigate the effects of abdominal and thoracic constriction. First we adjusted a pair of ordinary corsets so as to limit as much as possible any abdominal movement while leaving the thorax as free as possible.

We then took records of the respiration with the apparatus described above both in the sitting and lying positions. The records obtained are shown in Figs. 9, 10, 11.

These tracings show that the interference with the abdominal movements caused by the corsets hampers the even expansion of the lungs and produces a remarkable type of periodic breathing. This is especially noticeable when the subject is lying down. We have tried the converse experiment of leaving the abdomen free and constricting the upper part of the chest with a bandage. We did not obtain any very satisfactory results by this method because the bandage always slipped or stretched and so did not maintain the constriction. We found however that if the bandage did remain firm for a few minutes that the effect was to cause excessive periodicity of the respiration, periods of apnœa alternating with periods of very violent hyperpnœa.

In any condition such as bronchitis, asthma, or emphysema, in which the even distribution of air in the lungs is hindered by local narrowing of bronchi or local impairment of the lung elasticity, imperfect oxygenation of the mixed arterial blood must tend to be produced from the same cause as in shallow breathing. When shallow breathing resulting as a secondary nervous effect of the anoxæmia is superposed on the mechanical hindrance to even distribution of air the anoxæmia must become much more serious. An entirely new light is thus thrown on the symptoms. It is also evident that there is a very wide field for the application of continuous addition of oxygen to the inspired air as a means of relieving the symptoms and preventing the very serious secondary effects of anoxæmia. We hope, however, to discuss these matters in more detail in other papers.

## SUMMARY.

1. An apparatus is described which permits of regulated limitation of the volume of air inspired at each breath. This apparatus may also be used to obtain a continuous quantitative record of the rate and depth of respiration, normal or otherwise.

2. The effects of abnormal shallowness of the breathing were investigated and it was found that the shallow breathing causes uneven ventilation of the lungs and this in turn produces anoxæmia and consequently periodic respiration and other symptoms.

3. Observations were made on the effect of posture on the type and rate of respiration, and showed that the recumbent position is normally associated with slowing and deepening of the respiration and that if the deepening is prevented symptoms of anoxæmia are produced.

4. The effects on the respiration of abdominal and thoracic constriction were observed and recorded and were similar to those produced by the recumbent position.

5. These observations in conjunction with others recorded in this and a previous paper afford an explanation of orthopnœa.

The apparatus used in the work here described was partly provided by the Medical Research Committee and partly lent by Lt-Col. C. G. Douglas, M.C., R.A.M.C. and by Messrs Siebe Gorman & Co., Westminster Bridge Road, S.E. It was at the request of the Medical Research Committee to the British and Canadian Army Medical Authorities, and in consequence of facilities afforded to us by the latter, that the work was made possible. To all of these we wish to express our thanks.

## REFERENCES.

(1) **Breuer.** Stzngsb. d. Wien. Akad. math. nat. Kl. 2, Abt. **58.** S. 909. 1868.
(2) **Haldane and Priestley.** This Journal, **32.** p. 225. 1905.
(3) **Haldane and Mavrogordato.** This Journal, **50.** Proc. Physiol. Soc. p. xli; also **Haldane,** Organism and Environment. Yale University Press, 1916.
(4) **Head.** This Journal, **10.** p. 1. 1889.
(5) **Haldane.** Amer. Journ. Physiol. **38.** p. 20. 1915.
(6) **Yandell Henderson.** This Journal, **49.** Proc. Physiol. Soc. p. xxiv. 1915.
(7) **W. S. Miller.** Journal of Morphology, **8.** p. 165. 1893.
(8) **Pembrey and Allen.** This Journal, **32.** Proc. Physiol. Soc. p. xviii, 1905 and Med Chirur. Trans. **90.** p. 49. 1907.
(9) **Douglas and Haldane.** This Journal, **38.** p. 401. 1909.
(10) **Keith.** Further advances in Physiology, p. 182. 1909.
(11) **Christiansen, Douglas and Haldane.** This Journal, **48.** p. 244. 1914.

# THE PHYSIOLOGICAL ACTION OF EXTRACTS OF THE ELECTRICAL ORGANS OF THE SKATE (*RAIA CLAVATA*) AND TORPEDO (*T. MARMORATA*).

BY P. T. HERRING.

(*From the Physiology Laboratory of the University of St Andrews.*)

ELLIOTT(1) has given a number of reasons why the peripheral nerve cell of the autonomic system should be regarded as homologous with the protoplasmic nucleated mass at the nerve ending of a striated muscle fibre, and not with the nerve cells of the central nervous system or of the spinal ganglia. This view is based partly upon developmental grounds, and more especially upon the similarity of the reaction to nicotine and curari which is shown by both the motor nerve endings and by the sympathetic ganglia. Further the medullary cells of the suprarenals, and the chromaffin cells generally, may be considered as morphologically identical with the sympathetic ganglion cells. Elliott(2) points out that just as there is a close interaction between hormone (adrenaline) and nervous impulse in the sympathetic system, an interaction of a similar character may exist in other parts of the nervous system. It is indeed possible that the receptive substance of the motor nerve ending in cross-striated muscle contains a hormone, which, brought into activity by the nerve impulse, may work upon the muscle in a manner comparable with the action of adrenaline upon non-striated muscle. Obviously it is impossible to isolate and collect sufficient material from striped muscle to put this hypothesis to the proof. In the electrical organs of fishes however there is a great development of motor nerve endings with a minimum amount of modified muscular substance. An extract of electrical organs will therefore yield a material rich in extracts of modified motor nerve endings. It cannot be considered as identical with an extract of motor nerve endings of muscle because the electrical organs have undergone much change with the production of a tissue in which function is assumed by the action current in place of a muscular contraction. It was nevertheless considered worth while ascertaining if extracts of the electrical organs have any effect upon the contraction of cross-striated muscle. This line of reasoning was suggested to me by Dr Elliott, and at his request I have made the experiment.

Through the kindness of Professor M'Intosh I was able to obtain a supply of Skate (*Raia clavata*) in which the electrical organs are found as long cylindrical bodies on either side of the tail. Later on I was fortunate in securing a fresh specimen of *Torpedo marmorata*.

Extracts of the fresh electrical organs were prepared in Ringer's solution so as to be suitable for use with mammalian and frog muscle. Other material was dried, powdered, and similarly extracted. For control purposes extracts of similar strengths of fresh and dried skates' muscle were prepared.

The experiments were made with the isolated sartorius muscle of the frog immersed in a Lucas' muscle trough in the solution under examination. The muscle was stimulated directly by the break shock of an induction coil. Records were taken of the heights of the contraction of the muscle which resulted from stimuli varying in strength from minimal to maximal. The muscle was immersed first in extract of muscle and records obtained, then it was washed in Ringer's solution, immersed in extract of electrical organ, and another record of contraction taken.

No difference between the two extracts could be detected in the response of the muscle to electrical stimuli of the same strength. The height of contraction to the varying degrees of strength of stimulus was always the same whether the muscle was immersed in extract of muscle or in extract of electrical organ. There was no indication that the electrical organ contains any hormone which influences the contraction of cross-striated muscle. The experiment therefore fails to furnish any support in favour of Elliott's theory. The negative result does not invalidate the theory, however, for the reasons already mentioned.

Further experiments were made to ascertain if extracts of the electrical organs show any action upon other tissues. It is found that they exercise a slightly stimulating effect upon uterine muscle, but in this respect they are identical with extracts of skate muscle. Both give the same results when applied to the virgin uterus of the rat. The action is not a specific one, and may be accounted for by the well-known susceptibility of uterine muscle to foreign proteins.

Slight differences between the actions of the two extracts are found on perfusion of the isolated frog ventricle, but are not constant in different heart preparations.

The extracts were also tested upon the blood-pressure, kidney volume, and urinary secretion of an anæsthetised cat. Both produce a slight temporary fall of blood-pressure and are without influence upon kidney secretions. Extracts of the dried material show practically no result beyond a slight increase in kidney volume such as is occasioned by the injection of Ringer's solution alone.

Extracts of the electrical organ of *Torpedo marmorata* are much more depressant upon blood-pressure than are extracts of the electrical organ of the skate. The electric organ of the torpedo is very watery, and contains a preponderance of sodium salts (Weyl(3)). When dried and powdered it is very hygroscopic, probably because it contains varying amounts of magnesium salts. The dried extract of the skate's electric organ on the other hand shows little hygroscopic tendency.

## CONCLUSIONS.

There is no evidence that the electrical organs of fishes contain any hormone influencing the contraction of cross-striated muscle.

The negative results obtained with extracts of the electrical organs on cross-striated muscle give no support to Elliott's view that the autonomic ganglion cells are homologous with the motor nerve endings of cross-striated muscle, but they do not invalidate it.

Extracts of electrical organs show no special action upon non-striped muscle, cardiac muscle, blood-pressure or kidney secretion.

The expenses of this research have been defrayed by a grant from the Carnegie fund for research in the Universities of Scotland.

## REFERENCES.

(1) Elliott. Journ. of Physiol. 35. p. 438. 1907.
(2) —— Brain, 35. pp. 312 to 315. 1913.
(3) Weyl. Ztschr. f. physiol. Chem. 7. p. 541. 1883.

# THE SUPPLY OF OXYGEN TO THE TISSUES AND THE REGULATION OF THE CAPILLARY CIRCULATION.

By AUGUST KROGH.

*(From the Laboratory of Zoophysiology, University of Copenhagen.)*

In a previous paper I have deduced from the distribution and number of capillaries(1), the diffusion rate for oxygen(2) and the oxygen consumption of the various tissues that the oxygen pressure everywhere in the organism and especially in the muscles must be practically equal to that of the capillary blood, the pressure head necessary to maintain the oxygen supply being extremely low. This deduction does not agree with the results of certain earlier investigations of the oxygen pressure in the tissues of warm blooded animals, and it will be necessary therefore to try and clear up the discrepancies.

We can leave out of account as untrustworthy the determinations based upon the method of Ehrlich(3), as the power of tissues to reduce certain stains has very little to do with the presence or absence of free molecular oxygen. As pointed out in my monograph on the respiratory exchange(4) (p. 77) the tension determinations on secretions (Strassburg(5), Fredericq(6)), which have usually shown that free oxygen is practically absent, are likewise untrustworthy, because oxygen is used up at a comparatively rapid rate (Pflüger(7), Krogh(4)) in the fluids in question, and there is definite evidence that in certain secretions (urine, Krogh(4)) the $O_2$ pressure is normally not much, if at all, lower than in the venous blood. The experiments of Tobiesen(8) show that any gas mixture (or pure gas) introduced into the pleural cavity of man will finally get an oxygen tension of 3–4 p.c., and similar results have been found for the abdominal cavity of animals. Though it must be admitted that the introduction of air into the body may possibly influence the circulation in the adjoining tissues and increase their oxygen supply, the results of the tension determinations support on the whole the contention that mammalian tissues generally possess a rather high oxygen tension.

This conclusion is borne out also for the salivary glands by the ingenious experiments of **Verzár** (9), but in the case of muscles **Verzár** found that their oxygen tension must be comparatively low and perhaps 0, since a diminution of the arterial supply (and pressure) of oxygen caused a distinct decrease in the oxygen absorption of the muscular tissue.

Working in this laboratory on the relation between the oxygen pressure in water and the oxygen consumption of a fish **Gaarder** (10) found that the consumption increased with the pressure even up to a whole atmosphere. The increase in consumption corresponded to the increase in physically absorbed oxygen, and **Gaarder** concluded that the diffusion from the capillaries into the tissues is incapable of maintaining a positive oxygen pressure. This conclusion like **Verzár's** is seemingly at variance with my countings of capillaries and calculations of the oxygen pressure head.

I was led to undertake the investigations detailed in this and the two preceding papers by a comparison of **Verzár's** result for muscles with the circulation rate determinations made on man by **Lindhard** and myself (11, 12). These show that during rest the venous blood is about 65 p.c. saturated with oxygen, while during work the utilisation of the oxygen is much increased and the $O_2$ saturation of the mixed venous blood may fall to 16 p.c. or thereabout. The corresponding $O_2$ tensions will approximate 35 mm. and 12 mm. respectively[1]. At the same time the oxygen consumption of the body is increased from 3 c.c. even to 30 c.c. per kg. per minute. During heavy work the percentage saturation of the whole blood can be taken to represent pretty nearly the conditions in the working muscles, and during rest the muscular gas exchange is usually below the average for the whole body. It cannot therefore be doubted that during work a much increased volume of oxygen diffuses from the capillaries into the muscles, while the available pressure appears to be considerably diminished, when with **Verzár** and **Barcroft** we take the $O_2$ pressure in the muscular tissue to be very low. The increase in the rate of blood flow through the capillaries and even a passive distension of them caused by the increased blood-pressure would obviously be of no avail to explain the discrepancy, and I was led to the conclusion that if **Verzár's** results were substantially correct the only possibility must be an increase in the number of available capillaries.

---

[1] That the utilisation of the oxygen in the blood is much increased during work has also been shown by **Verzár** (13) for the cat's gastrocnemius.

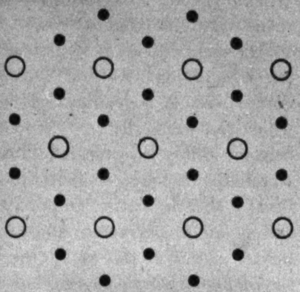


Fig. 1. Diagram of transverse section of muscle capillaries. ○ = open, ● = closed.

The conception to which I arrived was, that in the normal resting muscle a comparatively small number of capillaries arranged at fairly regular distances should be open, so as to allow the passage of blood while muscular work should cause the opening up of a larger number, thereby diminishing the distances which the oxygen molecules and other substances used by the muscle had to diffuse. This conception which is illustrated by the diagram, Fig. 1, has been put to the test of observation and experiment and found to be substantially correct.

### *Direct observations of the capillary circulation.*

1. The tongue of a frog (deeply narcotised with urethane) is pinned out and examined from the papillary side by transmitted light with a low power (binocular microscope, 65 diameters). Just after the pinning out the tongue is usually of a bright red colour and circulation is observed in a large number of papillæ. Later the tongue becomes pale, and circulation can be observed in the capillaries of a few papillæ only. Many are quite bloodless, but in some the capillaries are filled with blood which does not circulate. By local irritation with a needle circulation can be induced in any papilla from which the blood has disappeared, but not always in those in which the blood flow has stopped. In the course of about ten minutes generally the circulation thus set up becomes slower and slower, and finally the blood disappears anew from the capillaries under observation.

2. The geniohyoid muscles and the caudal portion of the hyoglossus are made accessible to observation by transmitted light by operative removal of the skin and the mylohyoids. The mouth of the frog is opened, the tongue drawn out and a small mirror is arranged in the mouth to illuminate the muscles through the mucous membrane. When the operation has been very cautiously made and bleeding is avoided the muscles are pale and circulation is observed in a few capillaries only, while some may contain blood which does not circulate. Tetanisation of

the muscles for a few seconds produces a greatly increased circulation (maximum usually after 15–20 seconds) and a large number of capillaries become visible. Afterwards the circulation is again diminished and may revert to the original state after 10–20 minutes.

On one occasion a single capillary was watched continuously after the stimulation. At first the red corpuscles passed in a continuous current, but a few minutes later they came one by one at intervals, which rose gradually to 5–15 seconds. At the same time the corpuscles became visibly transformed during their passage, being sausage shaped and distinctly longer than in the venule into which the capillary opened. Finally the circulation stopped completely, the capillary being empty and strongly contracted, and at that time no circulation could be observed in any of the visible capillaries supplying the same venule. In the venule itself there was, however, a slow current of blood with red corpuscles flowing one by one at distinct intervals, and this current was rhythmical and synchronous with the pulsations of the heart. I take this to show that one of the capillaries connected with that vein must have been open, the pulsation being accounted for by the absence of interference.

In another experiment of the same kind a record of the observations was dictated and the exact moments noted as follows:

Stimulation of nerve during 5 seconds.
After 24 sec. distinctly increased circulation.
„ 4′ 25″ corpuscles come through in a single file without intervals.
„ 4′ 55″ intervals of about the length of 5 corpuscles.
„ 5′ 25″ intervals increasing to length of 10 corpuscles.
„ 6′ 15″ corpuscles one by one or in small groups at long intervals.
„ 11′ 30″ no distinct alteration.
„ 13′ 10″ circulation in observed capillary stopped, but visible in others.

In a third experiment the average distance between the open capillaries of a resting *M. geniohyoideus* was estimated at 0·8 mm. After tetanisation it became reduced to 0·1 mm. The time of latency between tetanisation and the opening of capillaries has in several experiments been found to vary between 13 and 30 seconds.

3. As the observation by transmitted light is inconvenient and can only be applied to a few muscles I have tried observation by reflected light and found it to give excellent results on the muscles of the frog. As illuminant I have used direct sunlight or bright daylight, but the best results have been obtained by a small incandescent lamp (4 volt pocket lamp) mounted on a handle so that it could be brought very

close to the field of observation. By means of this arrangement I have examined a large number of muscles on many different frogs. The general results of these observations are as follows:

The capillary circulation in resting muscles is very variable, but as a rule feeble. It depends to a certain extent on the narcosis. A very deep narcosis will produce closing of a large number of capillaries and therefore bring out very strongly the changes following upon stimulation. The most normal results are usually obtained by injection of 0·2 to 0·25 gr. urethane on a frog of 35–40 gr. weight. The results obtained with this dose correspond closely to those obtained after injection of curari (1 mg. of an active preparation). Certain muscles (especially the *M. rectus abdominis*) are habitually well filled with blood, which appears to circulate in all or almost all the capillaries. (Average distance between capillaries about 60 $\mu$.) In most of the muscles of the trunk the circulation is feeble with distances between open capillaries varying between 200 and 500 $\mu$. In the limbs the circulation is often more feeble still, and sometimes it may be difficult to detect here and there an open capillary.

When the circulation in a muscle is not extremely feeble the open capillaries are generally seen to be arranged at fairly regular intervals.

By electrical stimulation or by gentle massage an increased circulation can be brought about in any muscle where it was feeble beforehand with the invariable result that the number of visible capillaries is greatly increased. Their average distances, observed in several cases, can be diminished to 60–70 $\mu$. By cautious administration of the urethane the respiratory movements of the floor of the mouth can be maintained while voluntary movements are suppressed, and in this case a fairly large number of open capillaries are observed in the contracting mylohyoids.

4. Similar observations have been made by reflected light on the muscles of urethanised guinea-pigs[1], but in this case special illuminating arrangements have been found necessary. I have used a small arc lamp and filtered the light through a dilute solution of methylene blue. The lamp was arranged to give a parallel beam of rays and by means of a concave mirror placed on an adjustable stand these were concentrated on the muscle under observation. The colour of the light caused the blood to appear very dark on a background of blue green. The platysma and prætracheal muscles proved to be especially well suited for these observations, though in some cases the vibration caused by the pulse in the carotids was very troublesome. The capillaries are too narrow to be readily seen by the greatest magnification which I found practicable

---

[1] The most suitable dose is about 0·8 gr. urethane to an animal of 400 gr. weight.

(binocular microscope, 65 diameters), but the observation is facilitated by the movement of the blood. Resting corpuscles are practically invisible. Just after the exposure of a muscle the circulation is feeble, the distance between those capillaries through which blood is flowing being about 200 $\mu$. While the muscle is exposed to air and the strong light the circulation is increased, and it is often possible to see the blood flow begin in one capillary after another. The distance between the capillaries may fall to 60 $\mu$ or lower, but reliable measurements cannot be made. When the muscle is moistened with saline and a cover glass applied, or when it is protected by the skin being drawn over it the circulation decreases again, but the distance between capillaries is usually not increased beyond 100–150 $\mu$.

Electric stimulation of a muscle for a few seconds always causes a considerable increase in the number of open capillaries, and in several cases spontaneous contractions have been observed in the platysma accompanied by a similar reaction on the part of the capillaries.

### *Capillaries in injected specimens.*

As I found it very difficult to obtain reliable measurements of the average distance between the open capillaries in living muscles I endeavoured to work out a method by which the condition at any given moment could be studied after fixation. I hoped to attain this end by injecting into the circulation of a living animal a suspension of particles which would be evenly distributed in the blood and by their presence indicate the capillaries through which the blood had been flowing. I tested a large number of different suspensions, mostly of small microorganisms, but none of these was satisfactory[1] and finally I had to have recourse to india ink. The particles in this fluid (Pelikan Perl Tusch. Günther-Wagner) are of ultra-microscopic size, and I greatly feared that they might penetrate into capillaries which were closed to the red corpuscles, but as far as I have been able to ascertain this does not take place. I have never seen a capillary containing india ink without red corpuscles. To the commercial preparation of india ink certain antiseptic substances have been added, and it was necessary therefore to dialyse for several days against a Ringer solution.

---

[1] Among the suspensions tested were fat globules from milk, some of which proved to be too large, while none of them could be stained deeply enough; small starch grains from rice, which were a little too large even for the frog and could not be stained satisfactorily; various bacteria, which became agglutinated; spores of Penicillium and finally the smallest known species of yeast (Saccharomyces exiguus) the cells of which could be stained deeply brown or black by silvering. The latter were not quite bad for experiments on frogs, but sometimes formed concretions which could not pass the capillaries. For much kind help and advice during these experiments I am indebted to Dr Vilh. Yensen of the University Pathological Institute, Professor of Anatomy, Dr F. C. C. Hansen, Dr Klöcker of the Carlsberg Laboratory and Mr Schmit Yensen of the Veterinary Serum Institute.

1. In the frog (*R. esculenta*) the injection is made through the vena cutanea magna[1] which is easily accessible from the ventral side through the abdominal portion of the *M. pectoralis*. About 0·5 c.c. of the suspension was usually allowed to run in from a micro-burette in the course of a few minutes. In most cases the frog was bled to a similar extent through the peripheral end of the vein, but I do not think this essential.

In the injected frogs the circulation could be studied directly under the binocular microscope, and the facts described in the previous section have been repeatedly verified on such, but in addition observations were made on excised portions of muscles and other tissues obtained after stopping the circulation by clamping the bulbus aortæ. These specimens were pinned on to small cork plates and stretched as accurately as possible to their natural dimensions. After being observed directly by reflected light they were fixed in 5 p.c. formalin and after the usual treatment finally mounted as microscope slides.

The observations of fresh material showed the differences between different muscles which have been described above. Muscles which had been tetanised before stopping the circulation were almost black and showed a large number of capillaries, while most of the others were pale and showed very few. The skin was always well injected. The tongue when not stimulated was generally white and nearly bloodless, and the empty stomach and intestines usually showed very slight signs of circulation only. The liver always became jet black.

In some of the mounted muscle preparations the number of injected capillaries per mm.$^2$ of the transverse section was estimated by counting. I thought at first that it would be impossible to make such countings on actual transverse sections of the muscles, because the injected capillaries are never uniformly black, but show numerous interruptions, caused chiefly by red corpuscles, and most of the countings have been made therefore on thick longitudinal sections or thin muscles mounted *in toto* according to the method described elsewhere (1). Countings of the capillaries in areas of 0·04–0·15 mm.$^2$ cannot of course give very accurate results, but the differences between resting and stimulated muscles come out very clearly nevertheless, and for approximate calculations of the necessary oxygen pressure head they are quite sufficient. Two countings of a stimulated sartorius have given 305 and 340 capillaries per mm.$^2$ respectively, while unstimulated but unusually well injected sartorii have given 85, 96 and 100. A stimulated extensor tarsi gave 195 capillaries, but in the corresponding unstimulated muscle the injected vessels were too few to be counted. There were certainly not more than five in a mm.$^2$. Three different preparations of the *M. rectus abdominis*, which is habitually vascularised, gave respectively 115, 155 and 180.

---

[1] I have also tried the *V. abdominalis*, but with the result that most of the injected substance was retained by the liver.

In the mucous membrane of the empty stomach and intestine the injection is very far from being complete, many capillaries being closed. The distance from any point in the tissue to an open capillary does not seem to exceed about 200 $\mu$ and seldom 100 $\mu$.

In the brain of the frog all the capillaries which are visible with total injection seem to become injected vitally also, and the supply of oxygen to any point in the tissue must be abundant—provided the diffusion rate for oxygen is not very much lower than in muscles.

2. On guinea-pigs of about 400 gr. weight I have usually injected 4 c.c. india ink through the jugular vein. Direct observation of several muscles after the injection confirmed the results obtained without injection. Considerable differences were observed between the degrees of vascularisation of different resting muscles, and on one of the animals the greater part of the muscular system appeared to have a larger number of open capillaries than usual. In this particular case the narcosis appeared to be less deep with occasional movements, but whether there is a causal connection between these phenomena I cannot tell. After killing the animals by blowing air into the jugular vein several muscles and other organs were examined more closely and from some microscope slides were prepared.

On the fresh muscles a number of countings were made of the capillaries visible in a single field with a low power and without using the vertical adjustment, and the positions of the capillaries on the micrometer scale were noted. By this simple method a general idea of the number of capillaries and the regularity of their distribution can be obtained. I give some instances of such countings.

Objective I. Magnification 90 diameters. 1 scale division = 22 $\mu$.
" III. " 230 " " " = 8·6 $\mu$.

Capillaries at scale divisions

Muscle from abdominal wall
Upper layer, Obj. I 10 13 16 21 21 24 28 31 33 35 38 44 50 55
61 65 67 72 75 78
Average distance 3·8 divisions = 83 $\mu$.
Deeper layer, Obj. III 29 32 35 40 42 47 52 54 56 60
Average 3·4 = 29·6 $\mu$.

Diaphragm, Obj. III ... 20 22 23 25 27 30 32 35 37 39 41 42 44 46
48 50 51 53 55 57 59 62 64 66 68 70 71 73
75 77 79 81 Average 1·96 = 17$\mu$.

Prætracheal muscle,
Obj. III ... 22 25 32 37 42 47 52 60 65 69 72
Average 5·0 = 43$\mu$.

These countings show a fairly regular distribution of open capillaries, which are however very different in number in the different muscles, being closest together in the diaphragm, which had been working vigorously up to the death of the animal, and widest apart in the upper layer of the abdominal wall. Some of these preparations have since been re-examined by the far more accurate method of taking optical transverse sections and have given the following results:

| | Section examined | | | | | |
|---|---|---|---|---|---|---|
| | Depth mm. | Length mm. | Area mm.² | Number of capillaries | Capillaries per mm.² | 2R $\mu$ |
| Abdominal wall Upper layer | 0·101 | 0·8 | 0·081 | 7 | 86 | 125 |
| | 0·095 | 0·8 | 0·076 | 6 | 70 | |
| | 0·081 | 0·8 | 0·065 | 6 | 92 | |
| Deep layer | 0·068 | 0·64 | 0·043 | 25 | 580 | 49 |
| | 0·095 | 0·48 | 0·045 | 22 | 490 | |
| Diaphragm | 0·054 | 0·29 | 0·016 | 43 | 2700 | 18 |
| | 0·054 | 0·145 | 0·0078 | 20 | 2550 | |
| | 0·058 | 0·262 | 0·0151 | 37 | 2450 | |

Optical sections showing the distribution and approximate diameters of the open capillaries in equal portions of three muscles with 200, 700 and 2500 capillaries per mm.² are given in Fig. 2. The results obtained by measuring the diameters of capillaries will be discussed below.

I have also tried a method of studying the state of the capillary circulation which should not involve any interference with the circulation. I have simply cut out small pieces of the muscles to be studied from the living animal (narcotised with urethane) fixed them in 5 p.c. formalin and stained 40$\mu$ sections with a weak solution of eosin. So far this method has not repaid the trouble spent upon it. A reliable differentiation of the red corpuscles

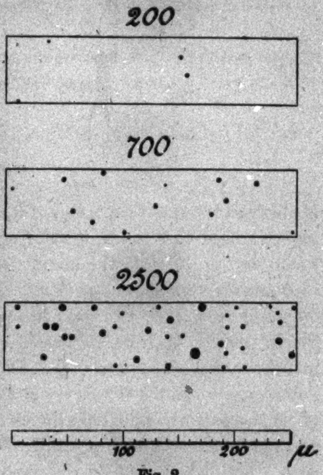


Fig. 2.

from the other histological elements could not be secured, though in a few cases they became quite distinct. Even if this difficulty can be overcome, as I think it can, the method has the drawback of not showing whether the blood in the capillaries has been circulating or stagnating.

### *The diameters of capillaries.*

In the preceding paragraphs the capillaries have been alluded to as being either open or closed, but this, though indicating the most important difference, is a very incomplete presentation of the case. The muscle capillaries which allow the passage of blood vary in diameter from about 2 to 10$\mu$, but in the resting muscle their average diameter is always very small viz. about 4·5$\mu$ in the frog and 3·5$\mu$ in the guinea-pig. The red corpuscles of the frog are on an average 22$\mu$ long, 15$\mu$ broad and 4$\mu$ thick in the middle, and those of the guinea-pig are 7·2$\mu$ in diameter and about 2$\mu$ thick. *A priori*, therefore, it seems almost incredible that they can pass through vessels of the dimensions given, but in the frog their actual passage can be easily observed in the living muscles under the microscope. The corresponding observation on the guinea-pig is difficult to make, but after injection of india ink the passage of corpuscles through extremely narrow capillaries can be seen distinctly, and mounted specimens of vitally injected muscles or of muscles stained with eosin, which can be observed and measured under the highest

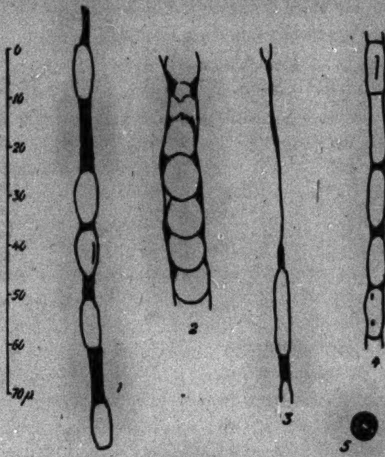


Fig. 3.

powers, leave no room for doubt. In the case of the injected vessels the presence of india ink between and along the corpuscles shows that they have been moving along during the life of the animals. Fig. 3 shows four different capillaries from the abdominal wall of a guinea-pig drawn by means of a prism. During their passage the red corpuscles become deformed according to the size of the capillaries. If the diameter of the vessel is about 4–5 $\mu$ (in the guinea-pig) the edges of the corpuscles are rolled in, as shown diagrammatically in Fig. 3, No. 5, and the diameter along the vessel is not materially increased, but in still narrower vessels the corpuscles become sausage shaped and their length can be increased to 14 $\mu$ or more (guinea-pig). The pressure necessary to bring about this change seems to be slight, but as soon as the corpuscles are released their shape becomes normal again. Their plasticity and elasticity are equally wonderful. When the corpuscles are much deformed, however, it is readily observed on the frog that their passage is comparatively slow on account of the resistance offered by them.

In working muscles a number of the capillaries show much greater diameters up to 10·4 $\mu$, and through these the corpuscles pass in a continuous current and without any change of form. Nevertheless the average diameter of the capillaries in the diaphragm of the guinea-pig was found to be only 5·0 $\mu$. In Fig. 2 the diameters of the open capillaries are recorded approximately but accurate measurements have not been made in this case.

In smooth muscles and in mucous membrane the capillaries appear to be on the whole wider, the corpuscles passing through with little or no deformation.

To show the variations in capillary diameters I give the following measurements in micromillimetres.

| | Frog, *M. sartorius* | | | | Guinea-pig | | | |
|---|---|---|---|---|---|---|---|---|
| | Resting | | Stimulated | | Abdominal wall | | Diaphragm | |
| | 7·3 | 5·2 | 7·2 | 7·4 | 2·2 | 4·0 | 4·4 | 4·2 |
| | 3·5 | 2·4 | 7·6 | 7·3 | 4·1 | 1·8 | 4·8 | 4·0 |
| | 2·5 | 4·1 | 6·0 | 8·0 | 3·0 | 3·8 | 7·4 | 2·8 |
| | 10·6 | 3·6 | 7·0 | 4·9 | 4·2 | 3·0 | 8·2 | 5·8 |
| | 2·9 | 4·3 | 7·6 | 4·2 | 4·5 | 3·5 | 3·3 | 5·4 |
| | 4·6 | 2·1 | 4·1 | 6·7 | 2·7 | 6·5 | 6·7 | 10·4 |
| | 5·6 | 2·7 | 9·6 | 6·7 | 3·7 | 3·1 | 2·2 | 3·5 |
| | 5·5 | 3·3 | 5·5 | 10·7 | 2·5 | 2·9 | 4·2 | 4·6 |
| | 4·0 | 2·9 | 7·4 | 5·6 | 2·9 | 5·0 | 4·7 | 5·5 |
| | 4·4 | 4·0 | 4·9 | 7·0 | 2·8 | 3·3 | 3·1 | 5·4 |
| Average | 4·3 $\mu$ | | 6·8 $\mu$ | | 3·5 $\mu$ | | 5·0 $\mu$ | |

### *The pressure heads necessary for the supply of oxygen to muscles.*

From the data given above and assuming the $O_2$ consumptions given below, column *a*, the pressure heads have been calculated for a small number of typical instances according to the formula given in another paper (1)

$$T_0 - T_R = \frac{p}{d}\left(1{\cdot}15R^2 \log\frac{R}{r} - \frac{R^2 - r^2}{4}\right).$$

They are recorded in column *e*.

In the frog the oxygen dissociation curve of the blood is very steep and the oxygen pressure in the veins and capillaries therefore very low[1] (about 2–4 mm.). With ten open capillaries per mm.$^2$ there must be lack of oxygen in considerable portions of the muscle, but with 90 the supply of oxygen is sufficient and a positive, though necessarily low, pressure must obtain everywhere. There can be no doubt from the observations that in resting muscles of the frog oxygen lack is a very frequent and probably normal occurrence.

| | *a* $O_2$ consumed per minute Vols. % of the tissue | *b* Number of capillaries per mm.$^2$ cross section | *c* $R$ $\mu$ | *d* $2r$ $\mu$ | *e* $T_0 - T_R$ mm. Hg. | *f* Total surface of capillaries in 1 cm.$^3$ muscle cm.$^2$ | *g*† Total capacity of capillaries Vols. % of the tissue | *h* Surface of 1 cm.$^3$ blood in capillaries cm.$^2$ |
|---|---|---|---|---|---|---|---|---|
| Frog muscle | | | | | | | | |
| Rest | 0·03 | 10* | 180 | 4·4 | 10 | 1·4 | 0·015 | 9500 |
| | 0·03 | 90 | 60 | 4·4 | 0·7 | 12 | 0·14 | 8500 |
| Work | 0·3 | 325 | 31 | 6·8 | 1·2 | 70 | 1·2 | 6000 |
| Guinea-pig muscle | | | | | | | | |
| Rest | 0·5 | 31* | 100 | 3·0 | 45 | 3 | 0·02 | 15000 |
| | 0·5 | 85 | 61 | 3·0 | 12 | 8 | 0·06 | 13000 |
| | 0·5 | 270 | 34 | 3·8 | 3 | 32 | 0·3 | 10500 |
| Massage | 0·5 | 1400 | 15 | 4·6 | 0·4 | 200 | 2·8 | 7000 |
| Work | 5 | 2500 | 11 | 5·0 | 1·4 | 390 | 5·5 | 7000 |
| Maximum circulation | 10 | 3000 | 10 | 8 | 1·2 | 750 | 15 | 5000 |

* These figures are taken from estimations of distances between capillaries ($2R$) on living animals. The corresponding values for $2r$ are from measurements on preparations with 90 (85) capillaries per mm.$^2$.

† For the calculation of the figures in column *g* the average sectional area of the capillaries measured has been utilised.

In artificial injections of fishes a very small number of the capillaries existing become injected. This must mean that the others were closed, and it is safe to conclude that here also the oxygen pressure is normally

---

[1] Unpublished determinations by Miss Leitch and the writer.

0 in very considerable portions of the muscles. This conclusion is confirmed by the experiments of Gaarder (10), mentioned above.

In the guinea-pig the oxygen tension of the venous blood is probably about 30 mm. With 30 open capillaries per mm.$^2$ there must be oxygen lack, but with 85 there will be a positive pressure everywhere, though it will be considerably below that of the blood. 85 is the lowest number of capillaries actually counted, but, as pointed out above, I believe from the observations made on living muscles that it is not really typical for the resting muscle, and I expect that further investigations will show the capillary circulation to be regulated so as to maintain the pressure at 0 in certain parts of the muscle in accordance with the conclusions of Verzár.

After massage and during work the oxygen pressure in the muscular tissue becomes practically equal to that of the blood, and it is evident that in these cases the circulation takes place through a much larger number of vessels than would be necessary to insure the supply of oxygen. It is therefore not improbable that the increase in number of open vessels may be brought about to meet other requirements of the muscle.

In column *f* I have calculated the total surface of the open capillaries in 1 cm.$^3$ muscular tissue. This is the area primarily available for diffusion and exchange of substances of any kind whatever between the blood and the tissue. When many capillaries are open this area is enormously increased.

In column *g* I have given the total volume of capillaries in per cent. of the tissue or, what amounts to the same, the percentage of capillary blood present in the muscles. When it is remembered that the total quantity of blood in a guinea-pig of 400 gr. is about 4 p.c. of the weight (14) and in a frog of 30–40 gr. about 4·5 p.c. (15) it is seen that the muscles contain during rest very little blood as compared with the whole organism. During work the amount of blood present in the capillaries of the working muscles is enormously increased. In the diaphragm of the guinea-pig it exceeds slightly the average for the whole body, and when the circulation in a muscle is at its maximum it may even rise to four times the average for the whole body.

### *The mechanism regulating the capillary circulation.*

As far as I have been able to ascertain the prevailing conception of capillary circulation among physiologists seems to be that the capillaries are passive, that blood is flowing continuously through all of them at rates which are determined by the state of contraction or dilatation of the corresponding arterioles, and that the dilatation of an arteriole will cause a rise of pressure in the corresponding capillaries, which will become passively expanded to contract again by their own elasticity when the pressure is reduced. It is obvious that such a view cannot be reconciled to the observations and experiments given above.

Some authors, who have observed that capillaries are sometimes bloodless, while the blood is flowing freely through a number of others around them, or that blood may be flowing through single capillaries, while the majority are closed, assume, that certain capillaries will not normally admit red corpuscles but only plasma (*Vasa serosa*) and that they are opened up when the pressure becomes high (Cohnstein and Zuntz, 1888(16)) or that "the capillary course from artery to vein offers various degrees of resistance" (Langley(17)). Langley adds: "It is probable that in the mammal there is a similar arrangement and that it is of considerable importance in shock, allowing some blood to pass to an organ when the blood-pressure is very low."

Such a view might explain a number of my observations on the assumption that the capillaries emanating from a single arteriole possess very different coefficients of elasticity and require very different pressures to be opened up. It should be pointed out, however, that if such an arrangement existed the blood would always flow through the same definite channels when the capillary pressure is low (as during rest in muscles) and the distribution of oxygen and nutritive substances would be very unequal, the cells lying nearest to the open capillaries being well supplied, while those at the greatest distance were starved. There is, moreover, abundant evidence to show that the capillaries are not merely passively distended by pressure but actively dilating and contracting.

This view has been maintained by a number of observers of whom Stricker(18), studying in 1865 the nictitating membrane of the frog, is, so far as I am aware, the first. Stricker's observations were followed up by Golubew(19), Tarchanoff(20) and, especially, Rouget(21) (1873–79) who confirmed the existence of contractile capillaries in the nictitating membrane and in certain fœtal and larval capillaries. Rouqet further described certain branched cells which were arranged on the outside of the contractile capillaries and which he considered as the contractile elements. These cells were again observed by P. Meyer(22) who claims their presence also on the capillaries of the bladder and gut of newts and frogs. By Mayer's results, Steinach and Kahn(23) (1903) were led to examine the contraction process more closely. These authors worked chiefly on the capillaries of the excised nictitating membrane of the frog and found that they showed spontaneous contractions and dilatations, though the internal pressure obviously remained at 0. They could induce contractions by stimulation of the dorsal sympathetic, and they were able to observe also spontaneous contractions in the capillaries of various organs taken from mammals.

A very important research, to which my attention has been drawn by Prof. Langley, was published as early as 1880 by Roy and Graham Brown(24). These authors studied the circulation in the web and other organs of the frog (tongue, mesentery, etc.), and they devised methods for measuring under the microscope the pressure in capillaries, small arteries and veins. They found that the capillaries studied show very frequent changes in diameter, and that capillaries belonging to the same arteriole may show simultaneous changes in opposite directions. They observed that when the pressure difference between the inside and outside of a capillary is reduced almost to 0 "the effect on the diameter is usually so slight as to be barely appreciable," while the vessel is at once emptied and collapsed when the outside pressure is raised, if ever so slightly, above the internal. Similarly a measurable increase in diameter cannot be induced by raising the inside pressure, but may come about spontaneously with a low as well as with a high pressure. The capillaries cannot therefore be more than slightly elastic but must possess active contractility.

Roy and Graham Brown have studied further some of the factors which produce active dilatation of capillaries and found that chloroform, mustard, etc., have a powerful action in this direction. They were unable to confirm the results of Severini(25) viz. that $CO_2$ causes dilatation and $O_2$ contraction of capillaries.

I can confirm from numerous observations the fundamental fact that capillaries do not contract measurably by elasticity when the pressure is reduced by closing or cutting the arteries, and I may add that the pressure necessary to force muscle capillaries open when they are actively contracted is very much higher than the normal arterial pressure as evidenced by the results of artificial injections. By such injections of fresh resting muscles nearly the same results may be obtained as from vital injections, viz., single capillaries are injected at long and more or less regular intervals. Only when all veins are tied and the pressure is raised to something approaching a whole atmosphere a complete injection can be obtained.

Especial importance attaches to the observation of Roy and Graham Brown that the calibres of capillaries are constantly changing. By the enormous number of available capillaries we can imagine the open channels as constantly shifting over the field and thereby producing a uniform irrigation throughout.

It should be mentioned finally that in recent years pharmacological evidence has been brought forward in support of the view that capillaries may react independently of the arteries and the arterial pressure. **Heubner** (26) has shown for instance that injection of certain salts of heavy metals ($AuCl_4Na$) causes an enormous number of capillaries to be opened up, of which no trace could be seen before the injection, while an action upon arteries could not be ascertained[1].

**Dale and Richards** (29) have brought forward evidence to show that histamin and adrenalin under certain conditions produce active dilatation of capillaries and "it is suggested that the current conception of the peripheral resistance to blood flow, as determined almost exclusively by the tone of the arterioles, allows too little importance to capillary tone as a factor."

That the mechanism regulating the calibre of capillaries is mainly situated in the capillaries themselves and must consist of contractile elements is, I think, established by the experiments and observations given above. The nature of the contractile elements, their innervation (sympathetic?, antidromic?) and their liability to influences from substances acting directly upon them must be a matter for further study, and though certain conclusions can be drawn from the observations recorded I shall reserve these for a future publication in which they can be put on a broader basis. Two essential points must however be touched upon.

1. By the direct macroscopic and microscopic observation of muscles and other tissues (mucous membranes of frogs) an intimate connection can be established between the colour of the tissue and the state of contraction or relaxation of the capillaries. When the open capillaries are few and narrow the tissue is pale; when they are numerous and wider the colour is more or less red. A muscle injected vitally with india ink can show all shades from a barely visible gray to black. The blood colour of a tissue is obviously not influenced at all by the velocity of the blood flow, but solely by the amount of blood *present*, and while the amount of blood present in the small arteries and veins is undoubtedly variable and must contribute to the colour, the variations are small compared with the variations in capacity of the capillary system. From the figures in column *g* p. 468 it follows that the maximum amount of blood which

---

[1] Several other substances have been claimed by **Heubner**, **Jacoby** (27) and **Holzbach** (28) to act specifically on the calibre of capillaries, but the evidence seems scarcely conclusive.

can be present in the capillaries of guinea-pig muscles is not less than 750 times the minimum. The blood colour of a tissue depends therefore practically on the state of contraction of the capillaries and not primarily on that of the arterioles.

This fact being grasped it becomes at once apparent that the capillariomotor regulation must play an enormous role not only in muscles, but in the skin and in all mucous membranes, in which changes in colour are the most obvious signs of vasomotor influences. The hyperæmic condition (in the clinical sense) of any organ is brought about by a large number of capillaries being open and more or less dilated, and in the anæmic condition most of the capillaries are closed, and most of those which remain open are more or less contracted.

2. The objection might be raised that the existence of a complete vasomotor mechanism for the capillaries would make the well-known arteriomotor mechanism rather superfluous. I have little doubt that the *current* of blood through a tissue could be regulated, at least temporarily, by the capillariomotor system alone when the arterioles were dilated, but this would involve a very high capillary pressure and probably give rise to exudation. The arteriomotor system regulates the *pressure* in the capillaries, and evidence can be adduced pointing to the simultaneous occurrence in the same tissue of contracted arterioles and dilated capillaries. I shall only mention as an example the well-known case of local hyperæmia with a more or less blue coloration. In such a case the blood must be flowing very slowly since the greater part of its oxygen has disappeared from the capillaries. A slow current through dilated capillaries must mean that the arterioles are strongly contracted.

## SUMMARY.

Microscopic observations, chiefly made by reflected light, are recorded to show that in the resting muscles of frogs and guinea-pigs most of the capillaries are in a state of contraction and closed to the passage of blood.

By tetanic stimulation of the muscles or by gentle massage a large number of capillaries are opened up. They can be observed to contract again afterwards. In spontaneously contracting muscles a large number of capillaries are likewise opened.

When the circulation is not extremely feeble (in the frog) the open capillaries are arranged at fairly regular intervals.

A method of *intra vitam* injection of india ink is described by which the capillaries which were open at the time become easily distinguishable in microscope slides prepared from the muscles.

The number of open capillaries per mm.$^2$ of the muscular cross section has been counted in such preparations of resting and working muscles.

The average diameter of the open capillaries in resting muscles is much less than the dimensions of the red corpuscles which become greatly deformed during their passage. In working muscles the capillaries are somewhat wider.

The oxygen pressure head necessary for the supply of the tissue has been calculated from the countings and measurements for some typical cases. The oxygen pressure in resting muscles is, sometimes at least, very low, but in working muscles it approaches very near to that of the blood.

The evidence, showing that the capillaries are not merely passively dilated by blood-pressure but constantly perform active changes in calibre, is discussed.

It is shown that clinical hyperæmia and anæmia are due mainly to changes in the calibre of capillaries and in the number of open capillaries.

Evidence is brought forward pointing to the arteriomotor and capillariomotor systems being able to act in opposite directions.

## REFERENCES.

(1) Krogh. Journ of Physiol. **52**. p. 409. 1919.
(2) Krogh. Ibid. **52**. p. 391. 1919.
(3) Ehrlich. Das Sauerstoff bedürfnis des Organismus. Berlin. 1885.
(4) Krogh. The respiratory exchange of animals and man. London. 1915.
(5) Strassburg. Pfl. Arch. **6**. p. 85. 1872.
(6) Fredericq. Arch internat. de physiol. **10**. p. 391. 1911.
(7) Pflüger. Pfl. Arch. **2**. p. 166. 1869
(8) Tobiesen. Deutsch. Arch. klin. Med. **115**. p. 399. 1914.
(9) Verzár. Journ. of Physiol. **45**. p. 39. 1912.
(10) Gaarder. Bioch. Zeitschr. **89**. 1918.
(11) Lindhard. Pfl. Arch. **161**. p. 233. 1915.
(12) Krogh and Lindhard. Skand. Arch. Physiol. **43**. p. 243. 1912
(13) Verzár. Journ. of Physiol. **43**. p. 243. 1912.
(14) Dreyer and Ray. Proc. Roy. Soc. Ser. B, **201**. p. 133.
(15) Fry. Quar. Journ. exp. Physiol. 7. p. 185. 1913.
(16) Cohnstein and Zuntz. Pfl. Arch. **42**. p. 303. 1888.
(17) Langley. Journ. of Physiol. **41**. p. 497. 1911.
(18) Stricker. Sitz. ber. d. Wien. Akad. d. Wiss. m. n. Cl. **51**. 1865.
(19) Golubew. Arch. f. mikr. Anat. **5**. p. 49. 1869.
(20) Tarchanoff. Pfl. Arch. **9**. 1874.
(21) Rouget. C. R. de l'Acad. der Sc. **88**. 1879.
(22) Mayer. Anat. Anz. **21**. 1902.
(23) Steinach and Kahn. Pfl. Arch. **97**. 1903.
(24) Roy and Graham Brown. Journ. of Physiol. **2**. p. 323. 1880.
(25) Severini. Ricerche sulla innervazione dei vasi sanguigni. Perugia, 1878. Quoted from Roy and Graham Brown.
(26) Heubner. Arch. f. exp. Path. und Pharm. **56**. p. 370. 1907.
(27) Jacoby. Ibid. **66**. p. 296. 1911.
(28) Holzbach. Ibid. **70**. p. 183. 1912.
(29) Dale and Richards. Journ. of Physiol. **52**. p. 110. 1918.

CAMBRIDGE: PRINTED BY J. B. PEACE, M.A., AT THE UNIVERSITY PRESS.

# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

## *July* 28, 1917.

### A high speed shaking machine.

By H. HARTRIDGE and G. WINFIELD.

A useful apparatus in the laboratory is the high speed shaking machine. Most of the designs which are at present obtainable commercially are of too light construction and suffer from too many (mechanical) defects to be capable of really efficient and prolonged service. Usually they possess some form of belt drive which slips or breaks in use. Their greatest fault lies in the entire lack of mechanical balance, with the result that the vibration of the entire apparatus is so great as to cause annoyance to other workers in the laboratory.

To avoid some of these defects we designed a shaking machine which embodies the best features of automobile engineering as evolved in the horizontally opposed twin cylinder engine. Since the rotating and reciprocating parts are individually and collectively balanced, the machine can be run at very high speeds with very little vibration. The machine which is shown in the accompanying illustrations is therefore suitable for the preparation of strong colloidal solutions in which very violent shaking is required. An advantage of the design is that the apparatus can be built up in any laboratory from commercially obtainable parts, since the crank-shaft connecting rods and flywheel are those of a Douglas Motor Cycle engine. The crank-shaft runs on caged ball bearings which are supported on suitable pillars. The bottles, which may be of any size found most generally useful in the laboratory, contain equal weights of fluid, and should be balanced against each other. If it is desired to use one bottle only for the solution, the second bottle should be filled with an equal weight of water. The bottles used in the apparatus illustrated held 750 cubic centimetres. In most laboratories Winchester quart bottles would probably be found more convenient. These bottles are given equal accelerations in opposite directions by means of the crank-shaft which is direct coupled to a one-sixth horse-power electric motor, through a simple universal joint. The bottles are held in well padded

steel cradles which are supported on trunnions. The bases of the trunnions on the left-hand side are placed closer together than the distance between their points of attachment at the top of the cradle. Those on the right-hand side, however, are placed further apart at the bottom than at the top. By this means a slight tilting motion is given to the bottles, which assists in causing thorough mixing and also helps the crank-shaft over the dead centres. A machine of this type has been found very successful in use. It ran at 750 revolutions per minute with very little vibration, the maximum velocity attained by the bottles being 660 feet per minute. Although the shaking is extremely violent calculation showed that the actual forces produced are not greater than an end pressure on the bottles of about 92 lbs. This pressure can be easily borne by ordinary bottles. The construction of the apparatus is sufficiently well shown in the photographs, not to require further verbal description. For smaller bottles the cradles can be supplied with baskets, into which they may be packed.

The motor, which was series wound for direct current at 220 volts, was found to drive the machine easily at full speed without becoming appreciably warm. A Resistance Box was provided however, so that slower speeds could be used if very violent shaking was not required.

## The histological structure of the moderator band of the sheep.

By Mildred Atkinson.

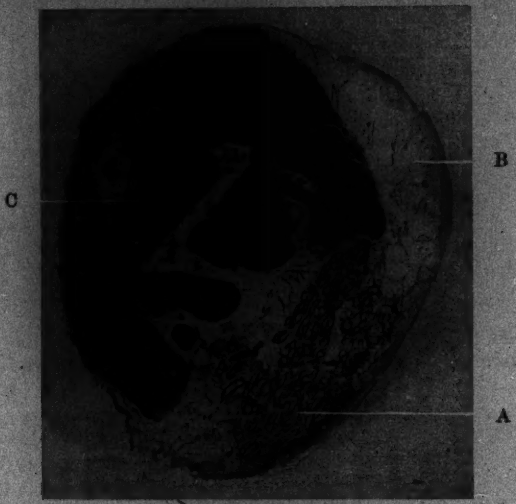
Purkinje fibres enter the moderator band from the inner ventricular wall in a well-defined roughly circular group. This group may be seen to turn sharply almost at right angles to its original course to run through the band. The group has a sheath of connective tissue, and in some cases fatty tissue, separating it from the muscle of the band.

The group of Purkinje fibres may contain perhaps twenty to thirty fibres on entering the moderator band, but owing to the branching of the fibres, the number increases as it approaches the outer ventricular wall, and the arrangement becomes more diffuse, as in photograph. On reaching the outer ventricular wall the mass of Purkinje tissue lies next the endocardium, and fibres run out from it in all directions into the heart muscle.

The group of Purkinje fibres is always accompanied by small nerve trunks, but in no case have nerve cells been seen.

Though as a rule the group of Purkinje fibres is definitely separated from the muscle of the band until the group approaches the outer ventricular wall, in certain cases a fibre can be seen leaving the group to run out into the muscle.

Besides this well-defined group of Purkinje fibres, individual fibres always lie among the muscle fibres which make up the major portion of the band. In the sheep a transverse section of the band shows that the group takes up one-sixth to one-tenth of the band, the remainder being ordinary heart muscle with a varying amount of fatty tissue. Actual connection between the individual fibres mentioned and the muscle fibres can be clearly made out in many sections, and this is also the case with the Purkinje fibres which run out from the group to the muscle of the outer ventricular wall.



T. S. Moderator Band of Sheep.

A. Purkinje fibres cut transversely.
B. Fatty tissue.
C. Muscle

Masses of connective tissue occur in the band associated with the insertion of chordae tendineae into the muscle. Inserted into these masses is found a peculiar type of heart muscle, of which the fibres are large, contain numerous nuclei, and stain only at the periphery.

The Purkinje fibre itself is of considerable length, consists of a central portion of homogeneous substance, assuming with Mallory's stain a purplish tinge, and showing at intervals unstained granular areas in which the nuclei lie, very often two together. The nuclei with Mallory's stain show up as bright pink in colour. The Purkinje fibres are pervaded with muscular fibrils which run roughly longitudinally in a reticular manner through the fibres. These show distinct cross-striation and on staining take on the same tint as the heart muscle itself. The Purkinje fibres are separated from one another by loose connective tissue, and surrounded by a delicate network of elastic fibres. Connection between two adjacent fibres is often made by means of side branches. The fibrillar substance can be made out to be continuous across the ends of two fibres.

# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

*December* 8, 1917.

## Demonstration of the Psycho-galvanic phenomenon.

By A. D. Waller.

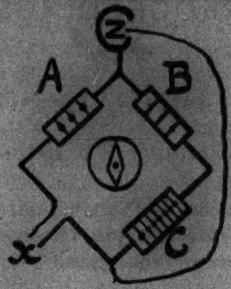
The present demonstration is in substance the repetition in modified form of a demonstration made to the Royal Society on November 8th.

Whereas in the previous demonstration subject, battery, galvanometer and calibrating resistance were arranged in series so that the resistance of the subject was to be obtained only by calculation from the deflection due to the calibrating resistance, in the present case the subject is placed in the $x$ arm of a Wheatstone's bridge so that the resistance is directly ascertained at the outset of observation by finding the resistance value for no deflection through the galvanometer. In this condition any emotive reaction of the subject is manifested by a galvanometric deflection from or near its zero point; the value of such deflection can be calibrated for photographic purposes, or directly estimated by comparison with a deflection caused by plugging or unplugging any convenient value at the resistance box. Gradual alterations of resistance during observation are conveniently ascertained by compensation.

When there is no current through the galvanometer the resistance of the subject $x = C\frac{A}{B}$ or simply $= C$ when $A = B = 1000$ ohms.

An emotive reaction causing a virtual diminution of resistance is directly measured by comparing the amplitude of the deflection with that caused by plugging out a given resistance at $C$, balancing at 27,000 ohms, *e.g.* (Photo No. 162) with a subject, the emotive response to a lighted match was 28 mm. The inspection value of the response was therefore 4000 ohms. The calculated value would be slightly less.

The high resistance of human skin to the constant current as compared with its much lower resistance to alternating currents, is due to polarisation. Gildemeister has recently (*Pflüger's Archiv*, vol. 162. p. 489. 1915) given a very elegant demonstration of the fact that the phenomenon consists really in a negative variation of the polarisation current. We may therefore simplify our calibration and avoid troublesome calculations by taking as our unit the volt in place of the ohm. The emotive reaction of 4000 ohms in Photo 162 is on the voltage scale a reaction of 0·4 volt.



# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

*January* 26, 1918.

## Further observations on the Thyroid-adrenal Apparatus. A histochemical method for the demonstration of adrenalin granules in the suprarenal gland. By W. Cramer.

In a short communication to the Society published two years ago I summarised a number of experimental observations which led to the conclusion that an increased activity of the thyroid gland leads to an increased liberation of adrenalin from the suprarenal glands, so that hyperthyroidism is in effect a slight but continuous hyperadrenalism. The conception was also formulated that the thyroid and suprarenal glands form part of a humoral or autacoid mechanism for the chemical heat regulation of the body. The experimental observations on which these conclusions were based have been confirmed and extended both experimentally and by a study of pathological conditions in man and animals. In the course of these observations which are based partly on the study of histological changes in the suprarenals, the necessity was felt for a method which would enable one to study the secretory activity of the cells of the suprarenal medulla in greater detail than is possible by the ordinary histological methods. The chromaffin reaction is a useful histochemical test for the presence or absence of adrenalin in the medullary cells, but fails to give reliable information as to the finer changes which one may presume to occur during the functional activity of these cells. Fixation by the ordinary fixatives (formol, sublimate, alcohol) not only fails to demonstrate the presence of the specific cell product (adrenalin), but when applied during the various stages of functional activity produces histological pictures which are misleading. Thus the Formol or Zenker preparations of a gland which has been stimulated to increased activity by the injection of β-tetrahydronaphthylamine ("$\beta$-N") show the medullary cells extensively vacuolated and apparently disorganised, while those of a gland which had been exhausted by exposure of the animal to cold, or by the injection of cultures of *vibrion septique* which rapidly kill the animal show the medullary cells well defined with sharp cell outlines, although rather smaller than the normal cells and with a denser protoplasm. Without reference to further tests one would be inclined to interpret the appearance of a gland with extensively vacuolated cells as exhaustion. Nevertheless these cells still give the chromaffin reaction and when tested by Folin's method are found to contain considerable amounts of adrenalin. Moreover the cells rapidly recover from this condition and resume their normal appearance. The truly exhausted gland, on the other hand, with its well defined cells gives the chromaffin reaction either faintly or not at all and does not appear to be compatible with the life of the animal. The readiness with which adrenalin reduces osmic acid suggested the use of the latter substance as a means to demonstrate the presence of adrenalin in the cells of the gland. If osmic acid is used in the form of vapour an excellent picture of the medullary cells with the adrenalin granules contained in them is obtained. The actual method used by us is as follows. The tissue enclosed in a wet gauze bag is suspended at 37° for $1\frac{1}{2}$ hours in a closed tube containing some 2 per cent. osmic acid solution. The tissue is then transferred to 50 per cent. alcohol, passed through the alcohols and imbedded in paraffin. The sections can be mounted directly in balsam and examined without further staining. In order to obtain good penetration thin slices of the gland must be cut with a sharp razor. In the case of the rat it is sufficient to halve the gland. In the mouse the glands are so small and the layer of cortical cells so thin, that the entire gland can be used after carefully removing the fatty tissue surrounding the gland. One obtains then a very good histological preparation of the entire gland with the medulla showing the adrenalin granules and the cortex with the lipoid globules. Both are stained black but can be easily distinguished from one another: the adrenalin granules are mostly very fine, jet-black and lustreless, giving the appearance of fine coal dust scattered over the medulla. Throughout the medulla there are, however, a few groups of cells, representing a different stage of functional activity, which have a clear and swollen appearance and in which the adrenalin granules are aggregated in a small black ball. The lipoid globules in the cortex are larger, round and the black has a glazed quality. The distinction can be made unequivocal by a device suggested to me by Dr J. A. **Murray**. The sections are placed in turpentine for 15–30 minutes after the paraffin has been removed with xylol. The turpentine dissolves all the blackened lipoids, but leaves the blackened adrenalin granules almost unaltered.

The method has been applied to the suprarenal glands of mice and rats subjected to the various conditions which, on the conception outlined above, were expected to produce alteration in the functional activity of the glands, namely: thyroid feeding, thyroidectomy, exposure to cold, injection of β-tetrahydronaphthylamine, injection of cultures of organisms of the *vibrion septique* type isolated from cases of gas gangrene[1]. In all these cases the method has given evidence of distinct changes in the medullary cells. It is intended to present a full report with figures illustrating these changes at a later date. But the following points may be briefly mentioned now: very clear evidence of the passage of adrenalin granules into the blood vessels of the medulla has been obtained in the case of glands stimulated to intense secretory activity by the injection of tetrahydronaphthylamine. Secondly the granules disappear if the glands are exhausted by such means as injection of *vibrion septique* or exposure to cold sufficient to reduce the temperature of the animal. Thirdly in the various conditions demanding an increased functional activity of the suprarenals fine black granules, similar to the adrenalin granules of the medulla and not dissolved by turpentine, appear in the cortex, especially in the layers of cells nearest the medulla. Whether these granules are actually adrenalin or whether they are perhaps to be looked upon as precursors of adrenalin it is at present not possible to say. This fact is however clear evidence that the cortex participates in the functional activity of the medulla and that these two parts of the gland are not two physiologically independent organs.

Staining of the adrenalin granules can also be obtained by a mixture of osmic acid solution and bichromate solutions, but the results are not as striking as those produced with osmic acid vapour.

After the death of the animal the adrenalin granules gradually disappear. In a mouse they are absent if the animal has been left for 20 hours after death in a warm room. They are still present although diminished in amount if the dead animal has been kept in the ice-chest for the same length of time. The method might therefore be elaborated for man as a medico-legal test.

---

[1] For these cultures I am indebted to Dr J. McIntosh of the London Hospital.

## The part played by an "accessory factor" in the production of experimental rickets. By Edward Mellanby.

In these experiments puppies of about two months old were fed on diets which were found to produce rickets in the course of 3–4 months. Two such diets were used:

(1) Milk, rice, oatmeal and sodium chloride,
(2) Milk and bread.

Salt is essential for life in the case of the first diet. The milk must be limited to less than 200 c.c. each day. If it is much increased no rickets develop.

In the case of other puppies, different classes of foodstuffs were added to the ricketty diet and the development or non-development of the disease investigated. The results were of such a nature that if rickets did not develop it could be assumed that it contained some anti-rachitic factor.

The following lists briefly indicate some of the results obtained:

| Substances preventing rickets | Substances not preventing rickets |
|---|---|
| (1) Meat. | (1) Protein of meat. |
| (2) Watery extract of meat free from protein. | (2) Casein. |
| (3) Malt extract. | (3) Linseed oil. |
| (4) Commercial yeast extract. | (4) 10 grams of yeast a day. |
| (5) 500 c.c. of milk each day. | |
| (6) Butter. | |
| (7) Margarines. | |
| (8) Cod liver oil. | |

An examination of these tables shows that fats, proteins and carbohydrates are not causative factors. On the other hand it seems clear that rickets is a deficiency disease of the type of scurvy and beri-beri. Similarly the anti-rachitic accessory factor has characters related to the growth accessory factor, although it is not identical with the latter, since rickets is rather an abnormality of growth and is most prominently shown in quickly growing animals.

Exercise may be an additional factor in the development of rickets but is not primary. Several dogs were kept on a mixed diet containing meat and confined without exercise for some months. They did not develop rickets. On the other hand puppies on a ricket's diet, however much freedom they are allowed, become lethargic in their movements some time before the legs bend. There is a physical flabbiness about them that one associates with rachitic children. When the condition is really developed no real exercise is possible owing to the physical disabilities.

To sum up:

(1) Rickets is a condition primarily due to the lack of an accessory factor in the diet.

(2) This accessory factor is found in (i) Extractives especially meat, (ii) Fats especially butter but not in linseed oil.

(3) Lack of exercise may play some part but not a primary part in the development.

# PROCEEDINGS
OF THE
# PHYSIOLOGICAL SOCIETY,
*March* 16, 1918.

### Histochemical observations on the functional activity of the suprarenal medulla in different pathological conditions.
By W. Cramer.

The functional activity of the suprarenal glands has been studied in mice by exciting the activity of the gland by the application of a stimulus such as the injection of tetrahydronaphthylamine into a number of young animals and examining the glands in half-hourly intervals by the histochemical method recently described[1]. It is found then that after the injection of a suitably large dose of the drug (2·5 milligrammes for a mouse of 20 grammes weight) there is during the first hour a massive secretion of fine black granules from the medullary cells into the blood vessels, during the second and third hours the medullary cells are almost completely depleted, but become filled again with fine black granules of adrenalin in the fourth and fifth hours. The rapidity with which the medulla is depleted is as striking as the rapidity with which it resumes its store of adrenalin. The secretory activity of the gland appears to be in itself a stimulus to the processes which lead to the new formation of adrenalin. If death occurs as the result of the injection, the mouse dies with laboured breathing and frequently with convulsions during the first stage of massive secretion of adrenalin into the blood stream. Post-mortem the lungs are found to be intensely congested and hæmorrhages can frequently be seen with the naked eye just as in animals which have died from toxic doses of adrenalin. Exposure to heat increases the symptoms and a dose which may be fatal to an animal kept in a hot room, may be borne without ill effect by an animal kept in a cool room. Larger animals (rabbits) die in hyperpyrexia.

The passing depletion of the medulla which occurs in this condition and which is associated with an increased secretory activity of the gland must be distinguished from the more lasting depletion which has been observed to occur as the result of infection with organisms of the vibrion septique type[1]. The depletion in these conditions (which is most readily explained as the result of an interference with the processes which lead to the new formation of adrenalin and which amounts to an exhaustion of the medulla), may be regarded as a factor contributory to the death of the animal.

An intermediate position must be assigned to a gland in which the medullary cells have become depleted as the result of the prolonged application of a stimulus, such as prolonged exposure to cold. Individual mice differ greatly in their resistance to cold. Under identical external conditions, involving the moderate application of the stimulus of cold, some animals remain well, while in others the temperature of the animal falls, and if exposure to cold is continued the animal dies. In the latter animals the medulla shows marked depletion of the store of adrenalin, while the medullary cells of animals which have remained in good health under the same conditions are fully charged with the fine black granules of adrenalin. Since in both groups of animals there must have been an increased secretion of adrenalin the difference in the condition of the medullary cells must be ascribed to the fact that in the animals which did not resist exposure to cold, the rate of adrenalin formation did not keep pace with the rate of adrenalin secretion. The death of these animals is clearly related to this failure on the part of the suprarenal gland. In the animals which did not succumb, the increased secretory activity of the medullary cells must have increased the processes of adrenalin formation to such an extent that the adrenalin store in the medulla was maintained. These results recall the observations of Elliott[2] and of Stewart and Rogoff[3] on the effects of splanchnic stimulation. These authors showed that splanchnic stimulation did not diminish the load of adrenalin in the medulla, although it produced a distinct secretion of adrenalin into the blood stream. In the striking experiments of Stewart and Rogoff the amount of adrenalin given off by the gland as the result of splanchnic stimulation during four hours was not less in amount than the load of a single gland. Nevertheless no deficiency in the store of the stimulated gland was found.

---

[1] Cramer, *Proc. Physiol. Soc.* January 26, 1918.

[2] Elliott, *Journ. Physiol.* **44**. p. 396. 1912.

[3] Stewart and Rogoff, *Journ. Exp. Med.* **24**. p. 734. 1916.

A severe hæmorrhage, uncomplicated by other conditions, produces within five minutes an active secretion of adrenalin which leads at first to an extensive depletion of the medullary cells. If the hæmorrhage is arrested and the animal recovers in a warm room, the active secretion of adrenalin continues, but the new formation of adrenalin also increases, so that adrenalin granules begin to accumulate again in the medullary cells within about an hour. The refilling of the medullary cells is facilitated by keeping the animals as warm as possible.

A small number of suprarenals have been examined of mice which died from post-operative shock within one-half to two hours after an abdominal operation during which the intestines had been exposed and handled. In this condition the suprarenal medulla presents an appearance somewhat different from that observed after a severe hæmorrhage. The medullary cells are more deeply vacuolated, but they are still fully charged with adrenalin granules and a very active secretion of adrenalin into the blood may be proceeding at the moment when the animal dies. The death of the animal is therefore clearly not due to exhaustion of the gland or to an inadequate secretion of adrenalin. Our results, which demonstrate an increased secretion of adrenalin into the blood in hæmorrhage and post-operative shock, confirm the conclusions of Bedford and Jackson[1], who demonstrated a greatly increased adrenalin content of the blood in these conditions by means of the biological test for adrenalin. The increased activity of the suprarenal gland in these two conditions must probably be interpreted as the response on the part of the animal to combat the low pressure which occurs in both these conditions. The significance of the difference in the appearance of the medulla in the two conditions is however not yet clear.

These observations also show, what has already been pointed out by Stewart and Rogoff[2], that the load of adrenalin in a gland represents merely the balance of formation and excretion, but gives no indication of the activity of the gland which is determined by the rapidity with which the adrenalin is built up and given off. The value of the histochemical method lies partly in the fact that it affords a means to study both the activity of a gland and its load at a given time without operative interference.

---

[1] Bedford and Jackson, *Proc. Soc. Exp. Biol. Med.* 13. p. 85. 1916.

[2] Stewart and Rogoff, *Journ. Exp. Med.* vol. 24. p. 734. 1916.

## The transport of carbon dioxide by solutions of sodium bicarbonate. By GEORGE A. BUCKMASTER.

How much sodium bicarbonate exists in the blood is unknown. Bohr considers that it is about 0·15 gramme per 100 grammes of blood. This salt is assumed to undergo dissociation in the blood, or be decomposed by some constituent or constituents of the blood while passing through the pulmonary capillaries. If this occurs then sodium bicarbonate can act as a means of transport for carbon dioxide.

The figures given by Léscouer show the dissociation tensions of solutions of sodium bicarbonate between 55° and 100° C. At the lower temperature the dissociation pressure equals 19 mm. Hg. My own experiments with varying quantities of pure sodium bicarbonate in boiled-out water were made at 38° C. The gas-space above the solution was either (1) air, (2) nitrogen, or (3) mixtures of nitrogen and carbon dioxide; the latter gas was obtained by heating solid sodium bicarbonate. Some of the results are given in the following tables. In the calculations the tension of water vapour at 38° C. is taken as 49·3 mm. Hg.

TABLE I.

Dissociation of $NaHCO_3$ in air at 38° C.

| Bar. P. | Grammes of $NaHCO_3$ | Hours at 38° | Dissociation pressure of carbon dioxide in mm. Hg. |
|---|---|---|---|
| 748 | 0·075 | 2 | 2·29 |
| 766 | 0·075 | 1·5 | 2·71 |
| 756 | 0·075 | 2 | 2·01 |
| 764 | 0·075 | 2 | 2·00 |

TABLE II.

Dissociation of $NaHCO_3$ in nitrogen at 38°

| Bar. P. | Grammes of $NaHCO_3$ | Hours at 38° | Dissociation pressure of carbon dioxide in mm. Hg. |
|---|---|---|---|
| 760 | 0·075 | 18 | 2·21 |
| 760 | 0·075 | 1·5 | 2·7 |

TABLE III.

Dissociation of $NaHCO_3$ in nitrogen and carbon dioxide at 38°.

| Bar. P. | Grammes of $NaHCO_3$ | Hours at 38° | Tension of $CO_2$ mm. Hg. | Carbon dioxide absorbed c.c. Exp. | Calcul. |
|---|---|---|---|---|---|
| 763 | 0·049 | 1·5 | 30·6 | 0·51 | 0·7 |
| 767 | 0·055 | 2 | 42·8 | 0·82 | 1·02 |

From these tables the average dissociation tension of sodium bicarbonate in air is 2·25 mm. Hg., in nitrogen 2·22 mm. Hg. With tensions of carbon dioxide within the range of the tension in alveolar air solutions of sodium bicarbonate do not dissociate at all; a solution of this salt actually absorbs carbon dioxide. Any assumed acid characters of constituents of the blood must necessarily possess stronger acid properties than carbon dioxide: for the actual existence of these there is no evidence[1]. From these experiments it is improbable that the transport of carbon dioxide in the blood is a function of sodium bicarbonate. || NB

### **Gum injections in "Shock."** By W. M. Bayliss.

A low blood pressure brought about by hæmorrhage, by injury to muscles, by temporary anæmia of a part of the body, by exposure to cold combined with slight hæmorrhage, by diminished oxygen supply as in rebreathing expired air, or by acid injection plus slight hæmorrhage, can, in all cases, be restored permanently by the intra-venous injection of 6 % gum acacia in 0·9 % sodium chloride. Sodium bicarbonate without gum has shown itself in my experiments to have only a temporary and comparatively small effect.

But this low pressure must not have lasted long enough to paralyse the bulbar centres by want of oxygen. When this has occurred, that is, experimentally, in about one and a half to two hours, according to the degree of anæmia, nothing avails, and transfusion of blood did not show itself to be superior to gum. Similarly, section of the spinal cord in the cervical region makes it impossible to produce a permanent rise of blood pressure by any kind of intravenous injection. Why this is so, is a matter for further investigation.

When injections of acid produce a progressive delayed fall of blood pressure, as they sometimes do under anæsthetics, or after moderate hæmorrhage, the effect is not due to the acid as such, but to some secondary changes. For injections of bicarbonate are of no value, whereas gum alone will restore the blood pressure. The direct immediate effects of acid are, of course, abolished by neutralisation of the acid.

As long as the respiratory centre is normally sensitive, as tested by carbon dioxide or vagus reflex, neutralisation of half the bicarbonate of the blood does not result in anything but an extremely minute increase in hydrogen-ion concentration; an increase of such a small degree as compared with those found experimentally to produce vascular dilatation, and so on, that it seems very doubtful whether it should be called "acidosis."

---

[1] Buckmaster, *Journ. of Physiol.* 51. p. 105. 1917.

If, after any procedure that would ultimately result in fall of blood pressure with "shock" symptoms, the arterial pressure be kept up by gum injections, the development of shock is prevented.

These recent experiments have led me to modify the point of view which previous experiments done in conjunction with Captain Cannon had inclined me to take. They compel me to look upon acidosis and its treatment as of secondary importance and the maintenance of an adequate supply of oxygen by a good circulation of oxygenated blood as the essential matter. This is attained more effectively by the injection of blood or gum than by drugs which constrict arterioles. A slight rise in hydrogen-ion concentration may, indeed, in some cases, be of advantage in bringing about a better arterialisation of the blood through the respiratory centre. By the copious supply of oxygenated blood to the tissues, acid products are oxidized and their further production retarded. Alkaline injections merely neutralize the acid without preventing the continued production of more acid.

So far as my experiments go, unless hæmorrhage has led to the loss of more than half of the total blood, there is no obvious advantage of transfusion of blood over that of gum, provided that the gum is introduced slowly.

### **Sudan III and the detection of fat.** By V. H. Mottram.

The well-known solubility of Sudan III and allied dyes can be utilised in the chemical laboratory for the detection of fat in a physiological mixture. In this it is preferable to the grease spot method and might well replace that test in class-work.

On solids the test is carried out as follows. One gram of the powdered solid is shaken with 10 c.c. of a saturated solution of Sudan III in 70 % alcohol for a minute. The fluid is filtered off through a small fat-free filter paper and the colour of the resultant compared with that of a control. The control is prepared by shaking one gram of a fat-free powder (such as starch or casein) with Sudan III solution and treating as above. When the tested solid contains more than 0·04 gm. of fat the Sudan III filtrate is markedly lighter in colour than the control.

To demonstrate the presence of fat in milk, the curd is precipitated with acetic acid, filtered on a small filter paper and washed with dilute acetic acid. The filter paper and contents are transferred to a test-tube, shaken with 10 c.c. of Sudan III solution and filtered as above. As a control, approximately the same amount of caseinogen as would be found in the milk is taken, moistened with dilute acetic and treated as in the test. The curd is coloured orange-scarlet as compared with the control, and the filtrate from it is of course lighter than the control filtrate.

That the loss in colour is very appreciable can be seen from the following experiments. Standard solutions of Sudan III in 70 % alcohol were made in the following strengths: full, half, one quarter, one eighth, one sixteenth and one thirty-second saturations. The precipitates from various quantities of milk were tested for fat by the given test, and the results were as follows:

1 c.c. milk + 5 c.c. $H_2O$ + 1 drop glacial acetic yield Sudan III filtrate in colour between $\frac{1}{2}$ and $\frac{1}{4}$ saturation.

2 c.c. milk + 10 c.c. $H_2O$ + 2 drops glacial acetic yield Sudan III filtrate in colour approx. $= \frac{1}{4}$ saturation.

3 c.c. milk + 15 c.c. $H_2O$ + 3 drops glacial acetic yield Sudan III filtrate in colour $< \frac{1}{4}$ saturation but $> \frac{1}{8}$.

4 c.c. milk + 20 c.c. $H_2O$ + 4 drops glacial acetic yield Sudan III filtrate in colour $< \frac{1}{8}$ saturation but $> \frac{1}{16}$.

5 c.c. milk + 25 c.c. $H_2O$ + 5 drops glacial acetic yield Sudan III filtrate in colour $< \frac{1}{16}$ saturation but $> \frac{1}{32}$.

A control with filter paper only gave a filtrate indistinguishable from full saturation.

A control with 1 gm. *commercial* casein gave filtrate < full saturation but $> \frac{1}{2}$.

The colour of the fully saturated solution is bright scarlet, and that of the $\frac{1}{16}$ and $\frac{1}{32}$ saturations pale pink; so that the fat in 5 c.c. of milk very appreciably modifies the colour of the filtrate, dissolving, on the least estimate, 69 % of the Sudan III in the saturated solution, and on the greatest, 94 %.

The test has been used in various classes in Liverpool, McGill and Toronto and has proved satisfactory in the hands of students. It is possible that a colorimetric quantitative method based on this test would prove of more value for some purposes than the quantitative methods of fat estimation already in our hands.

# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

## *March* 16, 1918.

### The lost time of the emotive response of the human subject.

By A. D. Waller.

The emotive response of the human subject associated with "feeling," consists in a sudden increase of conductivity of particular areae of skin, that especially of the palm of the hand, and it usually has a lost time of between 2 and 3 seconds, as illustrated by the accompanying record. The response to pain is uniformly greater than that to pleasure; to demonstrate the phenomenon in association with thought it is necessary that the thought aroused should be painful or alarming; the greater the pain or alarm the greater the galvanometric effect. The present record was taken upon a Belgian lady of considerable emotivity but of equally considerable inhibitory power over her thoughts, who had witnessed some of the worst horrors of the German occupation of Belgium (Termonde). She was able to keep her mind practically free of thought until my utterance of the word "Belgium" which was made simultaneously with the drop of a shutter, to serve as a signal of thought excitation. The interval of 3 seconds between excitation and cutaneous response, which at first sight might have been considered to be a cerebral lost time, is evidently a chiefly peripheral lost time, a time of elaboration taking place at the nerve terminals, presumably in the sweat glands of the palms of the hand; on this finding there is no considerable time required for the elaboration of the thought itself aroused by the word heard. The thought is practically simultaneous with its exciting cause.

The preceding conclusion is supported by measurement of the lost time of the emotive response to stimulation that cannot be regarded as producing "emotion" in the usual sense of the word, but which does actually consist in an emotor effect transmitted (to the muscles and) to the skin by motor nerves. The explosive discharge of a sneeze or cough are good instances of such emotor action free (usually) of any emotional quality.

Emotive response of G. de D. to the thought aroused by the word "Belgium"; the moment of utterance of the word was signalled by the passage of a shutter across the beam of light. The lost time of the response is 3 seconds; it lasts about 15 seconds. The scale to the left is in thousands of ohms; that to the right is in gemmhos (reciprocal megohms). The magnitude of the response is approximately $-2000$ ohms or $+6\gamma$.

Record No. 221 exhibits the emotive effect that accompanies a voluntary cough. The lower wavy line close to the time record is made by the shadow of a lever connected to a myograph fixed to my forearm, delicate enough to show the volume pulse and therefore delicate enough to show the slight movement that occurs with the cough. This movement, visible as a double spike caused by a double cough, serves to indicate the instant when the cough occurs, and the interval between this signal and the actual emotive (or emotor) deflection is found to be approximately 3 seconds; *i.e.* the discharge causing muscular contraction causes an electrical change in the skin (? sweat glands) of the palm of the hand 3 seconds later.

The rapidity of cerebral perception indicated by these observations, which at first sight seems incompatible with the ordinary doctrine of the comparative slowness of (cortical) cerebration, is in my view confirmatory of a measurement that I made many years ago to the effect that the shortest time required for an elementary act of discrimination is about equal to the time of a simple reflex act. A person with bandaged eyes

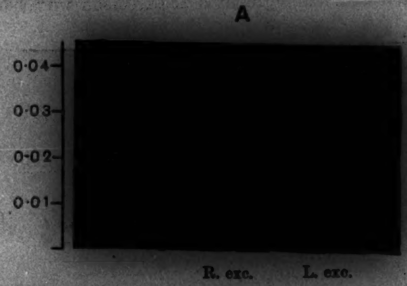
Emotive discharge of A. D. W. associated with a voluntary cough. (The galvanometer is twice as sensitive as in the previous record.) The moment of cough is signalled by the mechanical disturbance of the forearm. The lost time of the electrical change (increased conductivity) is 3 seconds. The magnitude of the change is greater than − 2000 ohms.

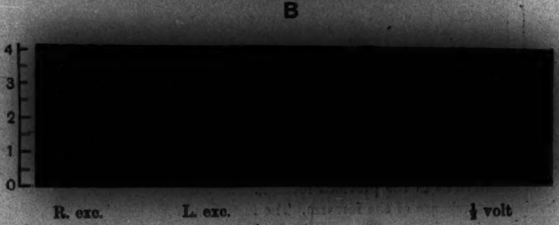
giving as the simple reaction time = 0·15″ to a touch on the hand, gives as the reaction time to *e.g.* a touch only on the thumb and not on the little finger, a shortest value of 0·30″, *i.e.* the shortest time of the higher level response with discrimination is double the time of the lower level response without discrimination. Complex or difficult cerebral discriminations may of course occupy any length of time, or may fail to materialise altogether.

## The alteration of conductivity produced in the pad of the cat's foot (post mortem) by excitation of the sciatic nerve. By A. D. WALLER.

At a meeting of the Society of Nov. 14, 1903 (*Journal of Physiology*, **30**) I demonstrated electrical (*i.e.* electromotive) effects produced in the pad of the cat's foot post mortem by excitation of the sciatic nerve. The facts were discussed in greater detail in my *Lectures on the Signs of Life*,

p. 97 et seq.; at p. 105 there are given two photographs by galvanometer and by electrometer showing that the latent period of the response to excitation of the sciatic nerve is 3 seconds. The voltage of the response in these two cases was 1 and 5 thousandths respectively; a very usual value in other cases has been $\frac{1}{100}$th volt. In a previous communication to the Royal Society (*Proceedings*, Nov. 21, 1901, **69**, p. 171) I mentioned the fact that a considerable augmentation of conductivity is produced by direct





Cat. 45 min. post mortem led off to the galvanometer by zinc discs covered with chamois leather moistened by normal saline, and applied to the pads of the two hind limbs. Excitation at R. and L. of the Right and Left sciatics. In *A* the responses are of the order of $\frac{1}{100}$th volt and are of opposite directions on the two sides (ingoing in each case). In *B* a current of 2 Leclanchés passes through the cat and galvanometer in series, the sensitiveness of the latter being reduced by shunt. Excitation of both R. and L. sciatics gives an increased deflection (due to increased conductivity) of which the voltage values are approximately for R. ⅓ volt., for L. ½ volt. The actual measurements are:

| | Resistance × 1000 ohms | Conductance in gemmhos |
|---|---|---|
| Before excitation | 62·5 | 16 |
| After " of R. | 50·0 | 20 |
| Before " of L. | 60·0 | 17 |
| After " " | 42·0 | 42 |
| At end of observation | 50·0 | 20 |

excitation, *e.g.* a rise from 21 at outset to 36 at close, and in the following year (*Proc. Roy. Soc.* **70**, p. 374) I made similar observations as regards the conductivity of human skin in consequence of direct excitation.

The present communication has as its object to demonstrate upon the cat during the second half hour *post mortem*, by excitation of the sciatic nerve, measurements of:

(1) the augmentation of conductivity;
(2) its lost time;

and to call attention to the essential similarity of these measurements with similar measurements taken on the human subject in connection with the emotive response.

The two records *A* and *B* are taken on the same cat $\frac{3}{4}$ hour post mortem by the same instrument unshunted (*A*) and shunted (*B*). The graduation which is the essential point to take into account in comparing the two records, is in both cases by voltage, the standard taken being $\frac{1}{100}$th in *A* and $\frac{1}{2}$ in *B*. The resistance in circuit was at outset rather over 60,000, at end about 50,000 ohms. The diminution of resistance at the excitation of R. sciatic was about 12,500, by exc. of L. sciatic 18,000 or preferably expressed in terms of conductivity a rise from 16 to $20\gamma$ and from 17 to $24\gamma$ ($1\gamma$ being the conductivity of a megohm).

The records now shown have been taken with an ordinary (Kelvin) galvanometer. The mechanical lost time of the instrument I used was measured to be $\frac{1}{8}$ sec. The lost time of the augmentation of conductance caused by excitation of the sciatic nerve was approximately 2 secs.

Records (unpublished) were taken in 1914 by my son W. W. Waller, M.B. of the lost time of the analogous effect obtained on the human hand by electrical excitation of its (motor) nerves at the bend of the elbow and just above the clavicle; the lost time was between 2 and 3 secs., there being no appreciable difference between the results of proximal and distal excitation.

# PROCEEDINGS
OF THE
# PHYSIOLOGICAL SOCIETY,
## *May* 18, 1918.

### Histological changes in nerve after compression without rupture of sheaths. By J. N. Langley. (*Preliminary communication.*)

In a previous paper[1] I have given an account of the degree of muscle atrophy and rate of nerve regeneration after compression of the nerve by a temporary ligature. In all these cases a histological examination was made of the nerves (peroneal and tibial). Since the nerves consist of several bundles, and the compression causes but little connective tissue growth, the bundles in one nerve can be isolated and treated by different methods. The time after compression at which the nerves were taken were 9, 21, 36, 38 and 48 days.

There were some notable differences in the histological appearances from those which have been shown to occur after nerve section:

1. After section, the central ends of the nerve fibres undergo for a variable distance Wallerian degeneration. In the compressed nerves this did not occur. The central end 9 days after compression was somewhat club-shaped, and the myelin stained more deeply than normal with osmic acid, hiding the axis if this remained, but it formed a continuous mass. In the cases of longer compression the appearance was not very different though the normal distinction of myelin sheath and axis became more obvious, there was no breaking up into globules and no solution of the myelin.

2. In preparations treated by Ramòn y Cajal's and Bielschowsky's methods one axis fibril only was found arising from each central nerve fibre. The same methods applied to cut nerves brought out numerous axis fibrils, some with recurrent fibres and spirals as described by Perroncito, Ramòn y Cajal and others in such cases.

---

[1] *Journ. of Physiol.* 51. 377. 1917.

I conclude then that Wallerian degeneration of the central end, and the formation of numerous irregularly coursing fibrils, are the result of injury to the nerves and of connective tissue growth and that they are not a necessary part of regeneration.

The formation of myelin was observed in the peripheral part of the nerve in osmic acid specimens. It began in the part of the nerve just below the compressed region, from this it developed upwards into the compressed region on the band fibres rapidly formed there, and downwards to the periphery. The extreme peripheral end of the nerve was unmyelinated for a considerable time after the myelin as a thin sheath had reached the central end of the nerve fibre.

Two other appearances which presented themselves require further investigation.

(1) In the proximal part of the regenerating nerve, the myelin was first visible on the outside of the band fibre and as this was much larger than the axis fibril, it was apparently not formed on the axis fibril. In this region only one myelinated nerve was formed. More peripherally there were sometimes two or three myelinated fibres in the same sheath, but the sheath may have been Henle's sheath.

(2) In the compressed region, there were rows of large roundish mononuclear cells between the fibres; these decreased to the periphery, and in their place were elongated masses containing myelin and neurokeratin granules. These appearances are practically the same as in severed nerves. But the phagocytes are generally supposed to be of the same nature throughout the nerve and the myelin in them to have arisen from dissolved myelin passing out through the neurilemma. The difference in appearance in the two regions seems to me to make it probable that the elongated masses are neurilemma cells which have been set free by breaking up of the neurilemma. If this occurs the neurilemma must break up in patches, for as is known some myelin globules remain closely attached to the band fibre up to a late stage.

# PROCEEDINGS
## OF THE
# PHYSIOLOGICAL SOCIETY,
## *June* 29, 1918.

**A simple respiratory stethograph.** By J. A. GUNN.

The device to be described for recording the respiration is cheap, can be quickly made from materials readily obtained, and gives a record of the respiratory movements (of the cat or rabbit) sufficiently accurate for many purposes. Fig. 1 illustrates the simplest form of it.

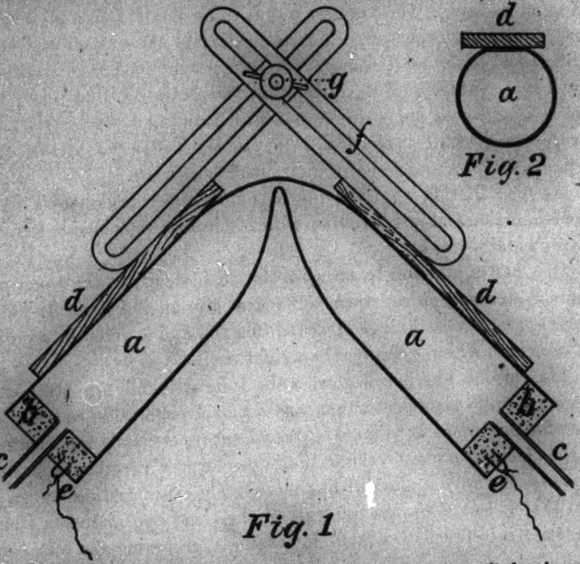


*Fig. 1*

*Fig. 2*

Fig. 1. Longitudinal section (diagrammatic) of Stethograph. *a*=rubber cylinder, *b*=cork, *c*=glass tube, *d*=wood-plate, *e*=staple, *f*=slotted plate, *g*=fly-nut.

Fig. 2. Transverse section.

*a* is a piece of rubber tubing 7 to 9 inches in length, 1¼ inches in diameter and $\frac{1}{16}$ inch thick. The rubber tubing sold for covering racquet handles answers well.

Two corks (*b*) are selected to fit tightly the rubber tubing, cut to about half an inch in length, and each bored and fitted with a short length of glass tube (*c*); one cork is inserted into each end of the rubber cylinder and the connection made tight with sealing wax.

Two pieces of thin wood or rigid cardboard (*d*) of length about one inch less than half the length of the rubber cylinder, and of width equal to the diameter of it, are fixed one on each half of the cylinder by telegraphic cement. Only the centre of the wood is attached (lengthwise) to the rubber (v. Fig. 2). The glass tubes (*c*) are connected by rubber tubing to a Y-tube and so to a manometer. It is preferable to have a side tube intervening between the Y-tube and the manometer for adjustment.

The apparatus can then be applied to the animal (anaesthetised and lying on its back). The simplest way is to fix small staples (*e*) into each cork, a string attached to which passes round the animal and can be adjusted suitably for tightness. The rubber cylinder of course bends to any angle required. With inspiration the flexible cylinder is pressed against the wood, air is driven out and the tambour lever rises. The lever returns with expiration owing to the resiliency of the rubber cylinder and the pressure of the tambour membrane.

Applied in this way, the respiration-recorder described is lighter, more sensitive and interferes less with respiratory movements than does Marey's stethograph.

It is better however to attach a slotted plate (*f*) to the wood plate, and the stethograph can then be fixed at any angle by the fly-nut (*g*). The apparatus can then be attached to a rod and lowered into position on the chest without resting the weight on it.

For routine pharmacological work I have found this stethograph more generally convenient than any form of respiration-recorder that I am familiar with. It probably gives a less proportionate representation of the changes in chest volume than does Cushny's stethograph, but it possesses the advantages of being more easily made, lasting longer, and also that it leaves the greater part of the chest and abdomen free for operative interference, such as, for example, recording the intrapleural pressure.

Fig. 3 shows a record taken by it of a familiar experiment—the effect of cutting the vagi on the rate and depth of the respiration. A simultaneous record was taken of the blood pressure and the intrapleural pressure. It can be seen by the tracing that the stethograph-record (*R*) indicates well the usual changes in respiration, and also that there is a sufficiently faithful correspondence between the respiratory movements and the consequent intrathoracic pressure. The stethograph tracing could of course have been magnified if desired.

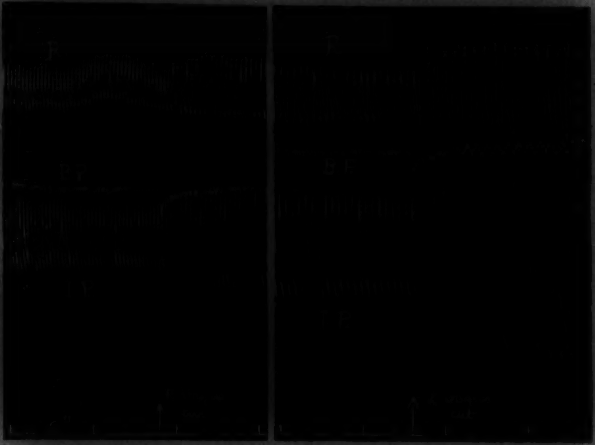


Fig. 3. Rabbit under ether anaesthesia. *R* = stethograph record of the respiration, inspiration recorded by upstroke. *B.P.* = Blood pressure. *I.P.* = Intrapleural pressure, negative pressure (inspiration) recorded by downstroke. Showing the effect of cutting the right vagus and (one minute later) the left vagus, on respiration, blood pressure and intrathoracic pressure.

## **On the concentration of antitoxic sera.** By ANNIE HOMER.

The addition of cresylic acid or "trikresol" has been recently advocated as an alternative to the adjustment of the reaction of plasma prior to its concentration by the Banzhaf (1913) and the Homer (1918) methods. It was found that, although cresylic acid consists of arbitrarily chosen distillates from coal tar, its percentage addition remained constant for the samples used during 1916–1918. However as only half the amount hitherto used is now required with a recent purchase of the substance, it is advisable to ascertain experimentally, for each consignment, the minimum amount necessary to produce clear end products. Should this procedure not be convenient then use can be made of phenol, a substance which can be easily obtained chemically pure.

A study has been made of the influence of phenol on the concentration of sera by my method. It was found that the increased precipitation of the serum proteins induced by phenol was regulated by its concentration in the plasma, by the reaction of the plasma and by the temperature at which the heating was conducted. At 58° C. the increased precipitation was practically the same for concentrations of phenol between ·2 and ·3 per cent. The addition of more phenol caused a further increase in the precipitability of the proteins; the optimum conditions for the agglutination and aggregation of the thus denaturated protein were not obtained unless the reaction had been previously adjusted or until the percentage of phenol had been raised to ·45–·5. For this reason, while clear end products were obtained from sera containing ·2–·3 per cent. and from ·45–·5 per cent. of phenol, those from sera containing from ·3–·45 per cent. were slightly opalescent. The former remained clear on standing and on dilution with saline; whereas, under the same treatment the opalescence of the latter was increased.

It was found that the percentage of phenol in plasma could be raised to ·5 per cent. without leading to an appreciable destruction of antitoxin or to its excessive precipitation with the First Fraction precipitates, or to an appreciable increase in the albumin content of the end products.

I have come to the conclusion that the preliminary adjustment of the reaction of plasma to *P* 8·3 (faint green tinge shown in 10 c.c. of plasma containing 3 drops of a ·1 per cent. solution of *a*-naphtholphthalein) gives rise to more reliable results than those accruing from the use of phenol or of cresylic acid, for, even in the latter case, the clarity of the end products is, in part, dependent on the reaction.

For those who cannot conveniently adopt the former method of treatment and who find that with the latter the end products, even after long standing in the cold room before filtration, tend to become cloudy, there is still open the previously suggested expedient of treating the Second Fraction precipitates with brine (Homer, *Biochem. Journ.* **11**. 21). In those laboratories where the First Fraction precipitates are also extracted with brine the duplication of labour thus involved can be obviated as follows:

The plasma—30 per cent. ammonium sulphate mixtures, after being subjected to the heating processes advocated in the Banzhaf and the Homer methods, are not filtered at this stage but are allowed to cool to room temperature when their ammonium sulphate content is brought up to 50 or 46 per cent. of saturation (as preferred) in the usual way. The liquids are then filtered and the precipitates, which thus comprise both the "First" and "Second" Fraction precipitates, after having drained thoroughly are well macerated in a volume of brine 1½ times to twice that of the original plasma; solid salt is added to ensure saturation. The brined extracts are subsequently filtered, to the filtrates are added 0·30 per cent. of glacial acetic acid and the ensuing precipitates filtered are pressed and dialysed in the usual way.

For the production of clear end products the brined liquids, after saturation with salt, must stand at room temperature for at least 3 days to allow of the flocculation of salt insoluble protein in a filterable form; this can be accelerated by warming the liquids to 45° C. before filtration. For the satisfactory recovery of antitoxin the brined liquids should stand for at least 6 days before filtration. From previously discussed work on the precipitation of heat denaturated protein by sulphates and its subsequent extraction with brine it follows that, while the process thus outlined leads to the production of clear products a complete recovery of antitoxin cannot be achieved unless the precipitates are subjected to a further prolonged extraction with a large volume of brine.

The potency of the end products thus obtained was about 8 times that of the original plasma; they contained about 40 per cent. of the original serum proteins. Those from the concentration of the same plasma by the Homer method showed a concentration of 10 times and contained only 25–30 per cent. of the original proteins. The former products contained appreciably more albumin than the latter.

### A note on the nature of the proteins associated with antitoxin in antitoxic sera. By Annie Homer.

A study of the gradient of the curves representing the precipitation of the serum proteins at progressively increasing percentages of saturation with ammonium sulphate indicates that there are no critical points for the precipitation of the eu- or the pseudoglobulin fraction or of the serum albumins. Thus, while the precipitation of euglobulin is not complete until the addition of ammonium sulphate has reached 33 per cent. of saturation, the precipitation of pseudoglobulin begins at 28 per cent. and is not complete until 44 per cent. of saturation has been reached. In the same way the precipitation of albumin begins at about 56 per cent. of saturation and is not complete until the solutions are practically saturated with ammonium sulphate.

An analysis of the observations made during a long series of experiments on the concentration of antidiphtheritic and antitetanic sera has shown that, while the respective antitoxins are associated with the pseudoglobulin fraction, the proportion of antitoxin associated with the proteins precipitated by ammonium sulphate in the above referred to ill-defined euglobulin-pseudoglobulin region is more considerable in antitetanic than in antidiphtheritic sera.

In antidysenteric sera the association of antitoxin with the proteins precipitated in the euglobulin-pseudoglobulin region is still more marked, in antimeningitic sera only a small fraction of the antitoxin is associated with the pseudoglobulin fraction, the main part is apparently attached to the euglobulin constituent of the serum.

Attempts to concentrate antidysenteric and antimeningitic sera by methods involving a heat denaturation of the serum proteins will therefore lead to considerable losses of antitoxin.

## **On the toxicity of cresylic acid.** By ANNIE HOMER.

The subcutaneous injection of solutions of cresylic acid into mice and into guinea-pigs was followed by the rapid onset of symptoms similar to those induced by the injection of solutions of phenol.

The acuteness of the symptoms and subsequent death of the animal were found to depend not only on the amount of cresylic acid administered but also on the composition of the sample and on the reaction of the solutions used.

It was found that, while in the control experiments the animals quickly recovered from the effects of the administration of a certain amount of cresylic acid dissolved in normal saline or in serum adjusted to $P_H$ 6·6–7·0, the symptoms became acute and even fatal where the reaction of the liquids had been adjusted to $P_H$ 8·3.

Where cresylic acid is added to antitoxic sera as an antiseptic, care should be taken to select ampoules and bottles of a suitable type of glass, for it is obvious that alkali dissolved from the glass by the serum will tend to accentuate the immediate discomfort due to the injection of the cresylic acid.

It was noticed that, with the animals under observation, the toxicity of the cresylic acid was more marked in solution in serum than in saline. Further, for a given dosage of cresylic acid in serum the symptoms were less acute with refined than with raw serum.

The toxicity of solutions of carbolic acid did not seem to be affected by similar adjustments of the reaction of the solutions prior to their injection.

## Note on the survival of the heart of the stale summer frog.

By W. L. Symes.

It was found by Ringer(1) that a perfusion fluid containing NaCl ·6 %, KCl ·0075 %, $CaCl_2$ ·01 %, $NaHCO_3$ ·01 % in distilled water, was inferior, during summer, to a solution containing NaCl ·6 %, KCl ·01 % in tap water, in its capacity to sustain spontaneous contractions of the frog's heart. The superiority of the tap water solution was attributed by him, in part, to the presence of calcium sulphate. He found later(2) that a solution containing NaCl ·6 % and KCl ·005 % in distilled water "saturated with $Ca_3(PO_4)_2$" was equally good in summer and in winter.

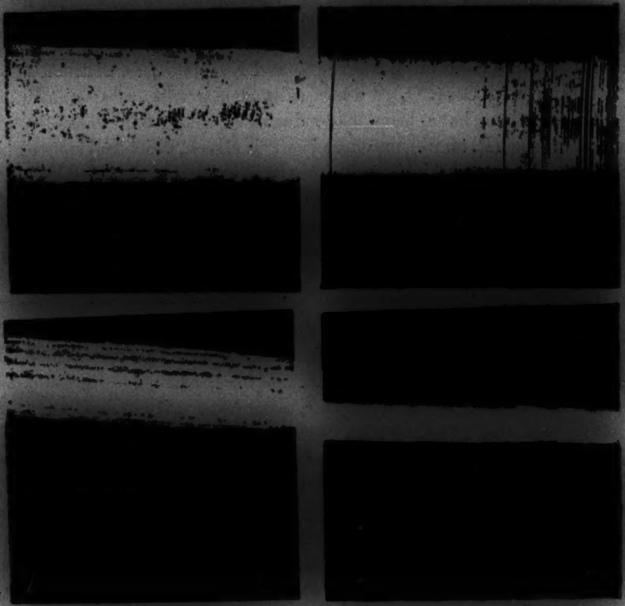
Observations have been made during the last five weeks on the hearts of frogs (R. temp.), purchased early in March, which had spawned in the laboratory and, which were below par in every noticeable detail. The hearts (auriculo-ventricular preparation) were perfused with the cannula described in 1911(3). Records were taken, of the heart beat by a light lever attached to the apex of the ventricle, and of the cardiac output by a recording siphon modified from Gunn's description(4) and arranged as demonstrated in 1916(5).

As would be expected, such hearts do not beat as well as or survive as long as those from frogs in better condition. The perfusion fluids (Locke-Ringer) have been either: *A*. NaCl 6·5, KCl ·42—·45, $CaCl_2$ ·24, $NaHCO_3$ ·20 ‰, in distilled water neutralised with $Na_2HPO_4$, with $CaCO_3$, with $Ca_3(PO_4)_2$ or with $MgCO_3$ or: *B*. NaCl 6·5, KCl ·45 ‰ in tap water neutralised with $NaH_2PO_4$. These two fluids have appeared to be equally serviceable. The addition of sulphate ($Na_2SO_4$, $K_2SO_4$, $MgSO_4$ or $CaSO_4$), to *A*, in concentrations exceeding millimolar has proved toxic. In lower concentrations it has seemed of doubtful value. A greater factor, influencing survival, is reaction. Slight deviation from neutrality, to neutral red, in either direction ($CO_2$, $NaH_2PO_4$, $Na_2HPO_4$, $Na_2CO_3$) has led to early irregularities and arrest.

Two such hearts were demonstrated to the meeting, 4–5 hours after preparation, perfused with Locke-Ringer solution containing NaCl 6·5, KCl ·45, $CaCl_2$ ·24, $NaHCO_3$ ·20 ‰ in each litre of distilled water neutralised in one case (upper tracing) with $Ca_3(PO_4)_2$, in the other with $CaCO_3$. The figure shows their behaviour during the second and fifth hours of perfusion.

The irregularities in rhythm which are conspicuous, during the fifth hour, in the upper tracing, were not observed in the lower, though the output (siphon record) shows more rapid failure. As compared with other hearts from the same group of frogs these two were slightly below average. As compared with hearts from fresh frogs they had failed, in the fifth hour, about as much as good hearts would have failed, under like treatment, in 25–50 hours.

It has appeared, from the foregoing observations, that the hearts of stale summer frogs are utilisable for several hours.



First and third lines show lift of lever. Second and fourth lines show discharges of recording siphons, 25 c.c. each. Fifth line shows time in hours.

## REFERENCES.

(1) This *Journal*, 6. p. 361

(2) *Ibid.* 7. p. 291.

(3) *Ibid.* 43. *Proc. Physiol. Soc.* p. xxv.

(4) *Ibid.* 47. *Proc. Physiol. Soc.* p. iii.

(5) *Ibid.* 50. *Proc. Physiol. Soc.* p. xxxiii.

## Further observations on the properties of antitoxic sera.

By Annie Homer.

The following points of interest have arisen from a study of the factors influencing the concentration of antitoxic sera by methods involving the fractional precipitation of the heat-denaturated sera by ammonium sulphate and by sodium chloride.

1. *The precipitation of antitoxin and pseudoglobulin from sera heated at a temperature of* 58° C. *for a period of from* 4–8 *hours.*

The extent of the heat denaturation of the proteins of antitoxic sera and of solutions of pseudoglobulin and antitoxin was regulated by the preliminary adjustment of the reaction of the liquids to values between $P_H$ 4·5 and 9·5. A comparison made between the extent of the precipitation of protein and of antitoxin by the addition of various percentages of ammonium sulphate to the heated and unheated liquids respectively has led me to the conclusion that:

(*a*) The extent of the increased precipitation of the serum proteins at concentrations of ammonium sulphate between 26 and 50 per cent. of saturation is a function of the denaturation induced during the heating.

Thus in unheated sera, the addition of ammonium sulphate to the extent of 28 per cent. of saturation, led to the precipitation of about 10 per cent. of pseudoglobulin; after a heat denaturation of about 47 per cent. had been induced in the serum, a similar addition of ammonium sulphate led to the precipitation of about 45 per cent. of the pseudoglobulin.

(*b*) The percentage precipitation of antitoxin with the First Fraction precipitates (30 per cent. of saturation with ammonium sulphate) may be regarded as being approximately proportional to the extent of the denaturation of the pseudoglobulin.

Thus, where the denaturation had been of the order of 24, 45 and 53 per cent. respectively there was a corresponding precipitation of 20, 40 and 50 per cent. of antitoxin with the First Fraction precipitates.

(*c*) An increase in the extent of the heat denaturation of pseudoglobulin is followed by a decrease in its solubility in brine. In such cases, even though the activity of the antitoxin thus precipitated with the salt-insoluble heat-denaturated pseudoglobulin is in no way impaired, the antitoxin is no longer in a condition suitable for use clinically.

(*d*) From antidiphtheritic sera in which a heat denaturation of about 30 per cent. had been induced the main bulk of the antitoxin was precipitated between 36 and 44 per cent. of saturation with ammonium sulphate.

(*e*) The further fractional precipitation of antitoxin and pseudoglobulin by ammonium sulphate in successive stages between the limits given in (*d*) did not lead to the separation of a fraction in which the ratio of antitoxin to protein was appreciably raised. Hence, as the antitoxin is thus evenly distributed over the proteins precipitated between the aforesaid limits it is clear that, in order to isolate antitoxin, means other than the fractional precipitation of the serum proteins must be employed.

2. *The relation between the denaturation of the serum proteins and the inactivation of antitoxin induced during the heating of sera at temperatures between* 56° *and* 75° C.

The extent of the denaturation of the serum proteins and the time of heating required for the maximum values to be reached were found to depend not only on the reaction of the plasma but also on the temperature at which the heating was conducted. As was anticipated the denaturation increased with a rise in temperature.

It was also found that, irrespective of the extent of the heat denaturation of the serum proteins, there was an actual inactivation of antitoxin during the heating of antitoxic sera.

While antitoxic sera could be heated to 56° C. for 15 hours or to 58° C. for 10 hours without any appreciable destruction of antitoxin, there were appreciable losses at higher temperatures. Thus, after 3 hours at 60·5° C. the loss was of the order of 10 per cent.; after 3 hours at 62° C., after 2 hours at 63° C. and after 30 minutes at 65° C. the losses were respectively 15, 25 and 33 per cent.; with a further rise in temperature the inactivation was appreciably greater in a relatively shorter time.

This inactivation of antitoxin by heat was not appreciably diminished by the addition of 2 per cent. of sodium chloride to either the plasma or the pseudoglobulin-antitoxin solutions previous to their being heated. Moreover, irrespective of the addition of sodium chloride, the losses at any given temperature were considerably increased by the addition of cresylic acid to the antitoxic liquids previous to their being heated.

In all cases the percentage losses of antitoxin induced during the heating of antitoxic plasma were greater than those experienced under the same conditions with refined antitoxic sera consisting of pseudoglobulin and antitoxin.

## A note on the influence of guanidin on the anaphylactic state.

By David Burns.

Smith (*Journn. Nerv. and Ment. Dis.* 1917, XIV. 26), from a clinical study of anaphylaxis, concludes that certain of the symptoms of this state are due to over-activity of the vagus. Burns and Watson (*Jour. Physiol.* LII. No. 2) have found that the administration of guanidin salts produces a block in the cardio-vagal inhibitory system.

From this it seems reasonable to expect, that the introduction of a regulated dose of a salt of guanidin, before or immediately after the administration of the toxic dose of serum to a sensitised animal, might remove or, at least, modify the anaphylactic symptoms.

Sixty-seven animals were employed.

(i) Guinea pigs were actively sensitised to horse-serum and, just before the administration of the toxic dose of serum, half of them were injected (i.p.) with 0·03 grm. per kilo of guanidin hydrochloride. All then received the toxic dose of serum.

Death rate per cent.

| Protected (guanidin) | Unprotected |
|---|---|
| 30 | 83 |

(ii) Rabbits were given a similar dose of guanidin *after* the anaphylactic symptoms were manifest.

| Protected (guanidin) | Unprotected |
|---|---|
| 0 | 75 |

(iii) Some rats were tried. In no instance did they exhibit anaphylactic symptoms. The post-mortem picture was invariably that of septic pneumonia.

| Protected (guanidin) | Unprotected |
|---|---|
| 30 | 94 |

The expenses incurred in this research were defrayed by a grant from the Carnegie Trustees, to whom I desire to record my thanks.

# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

*October* 19, 1918.

**On the creatin content of the muscles in rickets in dogs.** By P. S. Henderson.

The very striking loss of tone of the skeletal muscles in rickets has been described and discussed by Leonard Findlay in the Introduction to *A Study of the Social and Economic Factors in the Causation of Rickets* published in the Special Report Series of the Medical Research Committee, No. 20, 1918. He there points out that the "flaccidity of the limbs due to muscular weakness and laxity of the ligaments is very characteristic...."

"The muscular weakness may be so severe that the child is unable to support itself in either the sitting or standing posture" (p. 23). "In many cases the muscular weakness is extreme and seems out of all proportion to the severity of the osseous changes, so much so at times that one is constrained to believe that some myopathic condition is present. Electrical tests of muscles and nerves in rickets give normal readings...." "Henoch, Heubner and Zappert, in the absence of definite histological changes, are inclined to the view that the weakness of the muscle is secondary to disuse induced by pain in the limbs. Hagenback, Burckhardt and Bing, on the other hand, believe that it is the seat of a primary and specific dystrophy. Bing bases his opinion on microscopic changes in the muscles. He states that in all severe examples of the disease the muscular fibres are abnormally narrow, the transverse striation obscured, and the nuclei increased in number. Martins, however, in more recent investigations, cannot corroborate Bing's findings. It may be that it is disuse which causes the atrophy and weakness of the muscles, and that in part the paresis is of the nature of a reflex, to protect the painful parts against movement, but in view of Bing's researches further work is necessary before a primary implication can be excluded" (p. 26).

Pekelharing and van Hoogenhuyze (*Kon. Akad. van Wetensch. 6 Amsterdam*, **18**. No. 9, 1916) give analysis of the triceps of cats kept at rest in warm hutches from one to four weeks (seven animals) which show a decrease in the creatin content of the triceps brachii from 3·5 mgs., which they take as the normal in active cats, to 2·8 mgs. per 1 grm. of muscle, a fall of 20 per cent.

On the other hand after section of the sciatic nerve in cats, in spite of complete paralysis of the gastrocnemius muscles, we (Cathcart, Henderson and Noël Paton, *Journ. of Physiol.* **52**. No. 1, 1918) failed to find any decrease in the creatin content till after about 15 days, by which time degenerative changes in the muscle were well marked.

For the present investigation a number of pups from two litters were used. A full account of the observations will be published by Prof. Noël Paton, Dr Leonard Findlay and Mr Alex Watson. One series (five) were kept in the country and were normal. Another set (eight) were kept in the laboratory upon the same diet, and all became markedly rachitic as shown by X-ray and post-mortem examination. All the pups remained active, running about the laboratory except Dog 2 of the first set. In this the softening of the bone was so marked that towards the end of life it was unable to walk.

Those of the first litter were killed at eleven weeks, those of the second at thirteen weeks, by chloroforming and bleeding. The gastrocnemius muscle of the left leg was always used, and the creatin content was determined by the method of Brown and Cathcart (*Bioch. Journ.* **4**. p. 420, 1909). The results were as follows:

TABLE I. *Series I.*

| | Per cent. Creatin as Creatinine | |
|---|---|---|
| Animal | Normal | Rachitic |
| D 1 | 0·18 | — |
| D 2 | — | 0·12 |
| B 1 | — | 0·131 |
| B 2 | 0·18 | — |
| B 4 | — | 0·12 |
| B 6 | — | 0·15 |
| B 7 | — | 0·153 |

TABLE II. *Series II.*

| | Per cent. Creatin as Creatinine | |
|---|---|---|
| Animal | Normal | Rachitic |
| D 2 | 0·213 | — |
| | — | 0·134 |
| B 1 | 0·231 | — |
| B 2 | 0·235 | — |
| B 3 | — | 0·21 |
| B 5 | — | 0·102 |

TABLE III.

| | Average | Creatin Content | Percentage Difference |
|---|---|---|---|
| *Series I* | N | 0·18 | 25 |
| | R | 0·135 | |
| *Series II* | N | 0·226 | 34·5 |
| | R | 0·148 | |

N, Normal. R, Rachitic.

### CONCLUSIONS.

The very marked decrease in the creatin content of the muscles in rickets seems to point to some profound change in their metabolism which, when the activity of the rickety pups is considered, cannot be ascribed to loss of tone secondary to the bone changes.

I wish to thank Prof. Noël Paton for the help which he has given me.

The expenses of this investigation were defrayed by the Medical Research Committee.

# PROCEEDINGS

# OF THE

# PHYSIOLOGICAL SOCIETY,

## *December* 14, 1918.

### Persistence of the central somatic effect of strychnine after a large dose of nicotine. By J. N LANGLEY.

It is known that nicotine causes violent muscular contraction in vertebrates. This action in anæsthetised animals is due to stimulation of the various segmental motor centres though in the mammal the effect is fairly readily suppressed by excess of anæsthetics. In the skate I found (*Journ. of Physiol.* **27**. p. 232. 1901) that whilst local application of nicotine to the spinal cord caused purely local strong contraction, liberal application of nicotine did not prevent reflex contraction being obtained, though the contraction might be less than normal. Thus the ordinary reflex mechanism was not paralysed by a considerable amount of nicotine. Similar results of local application of nicotine were obtained by Dixon in the frog (*Journ. of Physiol.* **30**. p. 115. 1903), but apparently with a more distinct diminution of reflexes.

I have made some further experiments with a view of determining whether nicotine paralyses in the mammal any part of the central mechanism involved in somatic reflexes. The experiments were made on the anæsthetised cat. The blood vessels of one limb were tied, nicotine injected intravenously and a variable time after this, 2 to 5 mgms. of strychnine were injected. In no case did the injection of nicotine, even up to 100 mgms., prevent strychnine from causing clonic contractions in the leg with ligatured vessels. It follows then that nicotine has no specific paralysing action either on the anterior cornual cells or on the commissural cells on which strychnine is generally supposed to act.

The central somatic mechanism, however, soon ceases to be capable of stimulation by nicotine—just as is the case with the peripheral autonomic nerve cells of certain animals. Up to a certain concentration of nicotine there is stimulation with each increase; with further increase there is no stimulation but if the concentration is allowed to fall below a certain level, increasing it again will stimulate. On the theory of the existence of receptive substances the facts may be accounted for as follows: Stimulation occurs as long as nicotine is combining with the receptive substance; when complete combination has occurred there is no stimulation; whether stimulation is followed or not by paralysis depends on whether the completely combined substance retains its conductivity or loses it.

### **On a new accessory factor in bacterial infections.** (*Preliminary communication.*) By W. E. Bullock and W. Cramer.

The bacillus of Welch and the Vibrion septique, the organisms chiefly responsible for gas gangrene in wound infections, are by themselves non-pathogenic. Suspensions of these bacteria, whether obtained from surface cultures or from broth cultures by centrifuging and washing, do not produce gas gangrene when injected into mice, rats or guinea-pigs, and do not even produce any symptoms of ill-health. The normal animal disposes of these bacteria partly by lysis and partly by phagocytosis, and this defensive mechanism is so efficient as to render these bacteria non-pathogenic when they are injected by themselves.

If now a small quantity of a soluble ionisable calcium salt is injected together with a suspension of these bacteria, a violent gas gangrene is produced which kills the animal within 20 hours. With mice the smallest dose of calcium chloride necessary to produce this effect invariably is about 2·5 milligrammes, with guinea-pigs it is about 5 milligrammes.

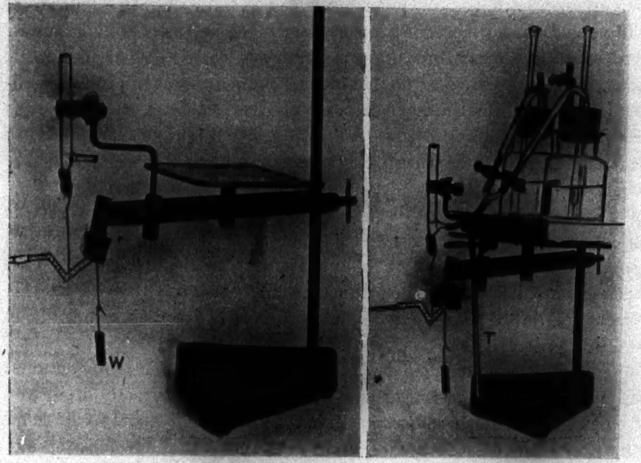
The same phenomenon is observed with the bacillus of tetanus. An animal injected with tetanus spores remains perfectly well, but if calcium chloride in the doses given above is injected at the same site or in a bigger dose at a different site, the animal develops tetanus and dies.

The chlorides of sodium, potassium, ammonium, strontium and magnesium are not capable of releasing the pathogenic properties of *B. Welchii*. The action of calcium chloride solutions can be antagonised by a solution of sodium citrate. It has also been possible to demonstrate an antagonism between Ca ions and Mg ions.

From a study of the mechanism of infection of the bacteria of gas gangrene the conclusion is drawn that calcium ions have the property of disturbing the relationship between the offensive mechanism of these bacteria and the defensive mechanism of the normal animal by which the bacteria are normally kept under control. The disturbance of this relationship leads eventually to a breaking down of this defensive mechanism. The term "kataphylaxis" is proposed to designate this new phenomenon.

### A student's apparatus for perfusion of the suspended frog's heart. By W. L. Symes.

The apparatus embodies several features shown, separately, to the Society during recent years. It consists essentially of a cannula, two reservoirs and a lever carried on a bar provided with a screw for moving the writing point towards or from the drum.



A B

A. One-fifth natural size, shows attachment of lever, of cannula, and of platform. The weight *W* partially counterpoises the lever.

B. One-seventh natural size, shows the apparatus complete except as regards the drain-tube *T* (see text).

The cannula remains as originally described (1), except that the inlet is lowered to 0·5 cm. above the outlet. It is inserted as follows. A thick wet, cotton (or flax) thread is passed under the aortic arches and behind the auricles as for the first Stannius ligature. After dividing the frenum the cannula is passed, *via* the sinus venosus, through the sinu-auricular aperture, into the right auricle and tied at the sinu-auricular junction. If the cannula is connected to a syringe (or burette), and the sinus distended during insertion, it (cannula) is easily passed into the auricle of a frog weighing no more than 20 grams. The heart is readily removed by a single cut (scissors) if lifted slightly by means of the cannula held horizontally. The arrest and subsequent slowing which follow ligation of the sinu-auricular junction are usually of brief duration. When prolonged, they are removable by a trace of adrenaline.

The reservoirs stand, preferably in a tray, on a platform clamped to the bar. They are ordinary wide-mouthed bottles (150–300 c.c.) each closed with a bung perforated for (*a*) a siphon outlet, (*b*) a Marriotte tube, (*c*) a vent, closed by a clamp. Siphon outlets are convenient owing to the small amount of horizontal space taken up by their connections. The upper ends of the Marriotte tubes are funnel-shaped to enable refilling without removing the bungs. This is done by (1) opening the clamp on the vent, (2) filling, (3) withdrawing air through the vent until the Marriotte tube is in action, and (4) re-clamping the vent. The connections between reservoirs and cannula are of the usual type. For the sake of distinctness only one drain is shown. In practice two are employed, one between each reservoir and the Y-piece, so that, either reservoir may be emptied and refilled during undisturbed perfusion from the other.

The lever consists of flexible metal, with a glass writing point, jointed to a V-shaped piece of celluloid with the bottom of which the apex of the heart is connected (see Fig.) by a thread. The fluid expelled by the ventricle passes down the thread and is guided by the point of the V into the vessel (*e.g.* a recording siphon) provided for its reception. Celluloid is advantageous, here, as it is not affected by salt solutions. A convenient magnification is 8–12-fold. The spindle carrying the lever bears a small pulley wheel for the attachment of a counterpoise.

Drugs may be applied to the heart (1) in dilute solution, from a reservoir, (2) in concentrated solution, from a pipette (or burette) discharging slowly into the cannula, (3) in solid form dropped into the cannula.

### REFERENCE

(1) This *Journal*, **48.** 1911–12, these *Proceedings*, p. xxv.

## Calibration of a dock labourer by means of his $CO_2$ discharge.

By A. D. WALLER.

By the courtesy of the Port of London Authority, and thanks to the facilities provided for me by Mr H. J. Deane, I have obtained hourly volumetric estimates at the Surrey Commercial Docks, of the $CO_2$ exhalation of two dock labourers during three successive days of fairly strenuous work (operations involved in the laying of a concrete floor, *i.e.* mixing stuff, wheeling barrows, shovelling, trowelling, ramming) from which conclusions are possible, but the principal value of which in my opinion consists in proof of the fact that by the comparatively simple procedure of measuring the $CO_2$ discharge alone, a "fair" estimate, *i.e.* an estimate within limits of ± 5 per cent. can be made of various kinds of work not directly measurable in mechanical units. My thanks are also due to the Resident Engineer, Foremen and Labourers who forwarded the task, and to Miss G. De Decker who carried out the hourly collection and measurement of samples.

*Method.* Expired air was collected at hour intervals throughout the working day for 1 minute or preferably ½ minute (by stopwatch) in bags (ordinary air pillows provided with suitable wide bore tubing, valves, and three way tap, capacity 30 litres). The volumes collected were measured by a Verdin Spirometer and the $CO_2$ percentage estimated at once by KOH absorption and displacement by acidulated water.

The results can be most succinctly given in graphic form giving the values of the $CO_2$ ordinates for the discharge during the half-minute immediately after work and during a half minute after 3 minutes of rest (standing).

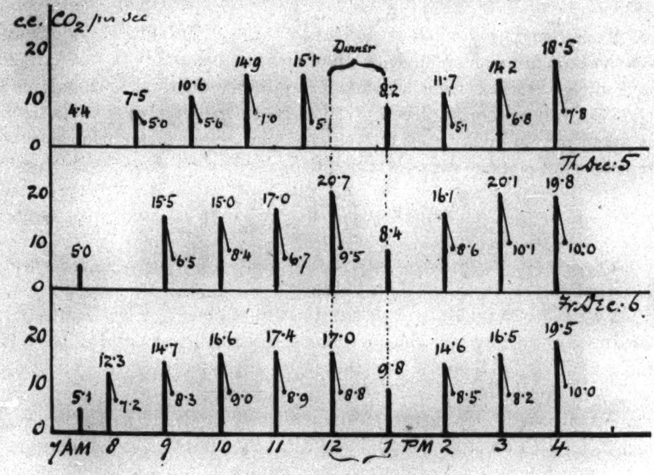
I learn from these data that an efficient dock labourer with a resting $CO_2 = 5$ c.c. per sec. does hard work (concrete floor laying) at an average cost ranging in the course of the day, 7.30 a.m. to 12 p.m., and 1 p.m. to 4 p.m., between + 10 and + 15 c.c. $CO_2$ per second.

The cost of work (assumed as having been uniform) expressed in $CO_2$ has been progressively greater during succeeding hours of work. It has been greater during the last three hours of the afternoon than during the last three hours of the morning. In Perry's case the averages of the three days have been:

| Morning (c.c.'s $CO_2$ per sec.) | | | Afternoon (c.c.'s $CO_2$ per sec.) | | |
|---|---|---|---|---|---|
| 10 A.M. | 11 | 12 | 2 P.M. | 3 | 4 |
| 14·10 | 16·44 | 17·60 | 14·12 | 16·94 | 19·28 |

and the $CO_2$ value of the day of 8 hours has been: 328·6 L. (= 1942 calories).

Cost of work expressed in c.c.'s $CO_2$ discharged, is in my judgment the essential and easily observed datum. It can of course be refined upon by further observation of $O_2$, and calculations by body surface in heat values that vary with the respiratory quotient. But I thought it more important to obtain for Perry's ease his $CO_2$ value for known work and did so on the following afternoon by means of staircase work.



Dock labourer. Perry. Hourly values of his $CO_2$ ordinate during 3 days laying of a concrete floor.

Saturday, Dec. 7th. Perry (weight, 83 kilos, height = 1·71 metre) made 10 ascents of the 20 metre staircase. At each ascent the expired air was collected for the last 10 metres (timed by stopwatch as done in 35 ± 5 secs.) in bag No. 1; and at the top of the staircase in bag No. 2 for 30 secs., with least possible delay.

*Results.* The summary of results is as follows, the figures refer to bag No. 1, those of bag No. 2 were on the average slightly higher.

| | |
|---|---|
| During work at 21·6 kgm per sec. | 25·8 c.c. $CO_2$ per sec. |
| At rest ... ... ... ... | 5·0 |
| Cost of work ... ... ... | 20·8 or 0·96 c.c. $CO_2$ per 1 kgm. |

$$\frac{\text{work}}{\text{cost}} = \frac{1}{0{\cdot}96 \times 2{\cdot}5} = 0{\cdot}43 \text{ on the assumption } 1 \text{ c.c. } CO_2 = 2{\cdot}5 \text{ kgm.}$$

According to these figures the mechanical value of the 3 days dock labour costing 10 to 15 c.c. $CO_2$ per sec. was at the rate of 10·4 to 15·6 kgm. per sec. or 0·14 to 0·21 Horse Power.

## The cost of mechanical work in terms of $CO_2$ expired. By A. D. WALLER.

Measurement by indirect calorimetry of the external work developed by the human machine depends upon measurements of $CO_2$ discharged and of $O_2$ absorbed, from which the respiratory quotient is calculated; the heat value per litre of $O_2$ (or of $CO_2$) corresponding to this quotient is taken out and converted into calories per square metre from curves representing Dubois' formula:

$$\text{Area} = Wt^{0·425}_{(\text{kilos})} \times Ht^{0·725}_{(\text{cms.})} \times 71·84^{1}.$$

A full account of the procedure has recently been given by Cathcart (*Journal R.A.M.C.*, November, 1918).

My present purpose is to justify the use of a shorter method that I used many years ago for the continuous observation of varying physiological activity and that I have taken up again recently in connection with work problems. It consists in the measurement of $CO_2$ discharge alone at frequent intervals from comparatively small samples of expired air, without $O_2$ determinations, but with the assumption (when required for computations comparable with the computations made by the longer method) of an average respiratory quotient (0·84 at which the mechanical equivalent of 1 c.c. $CO_2$ = 2·5 kgms)[2].

The graph of $CO_2$ ordinates observed at frequent intervals serves as an indicator of the rate at which carbon is being oxydised at the moment by the human machine, *i.e.* of its physiological activity.

The $CO_2$ ordinate can be observed:

A. In relation to known mechanical work measured in kilogrammeters. This implies a measurement of mechanical efficiency.

B. In relation to mechanical work of unknown value when we have ascertained by observations of type A at what cost of $CO_2$ known amounts of work are developed.

---

[1] Dubois and Dubois. *Russell Sage Reports*, vol. I, "Tenth paper on Clinical Calorimetry," p. 11.

[2] R. Q. =0·70, 0·74, 0·79, 0·84, 0·89, 0·94, 1·00.
KgM. =2·827, 2·735, 2·621, 2·506, 2·392, 2·277, 2·140.

The following notes of an observation of type A are given *in extenso* to illustrate the procedure, and to demonstrate its obvious convenience for the calibration of work not directly measurable in kilogrammeters. Observations of type B are in progress.

Nov. 26th, 1918, Cadet R. aged 18½; weight (clothed) 64·1 kilos, height 5 ft. 9 in.

Samples of expired air collected at intervals of 5 mins. before, during and after work consisting in ascents of a 20 metre staircase at intervals of 5 mins. Each sample was collected in a bag during the second half of each ascent, 10 metres, and the rate of ascent was taken by stopwatch. Estimates of $CO_2$ were made at once, and one $O_2$ estimate during the hour was made by Mr Symes, showing a resp. quot. = 0·82.

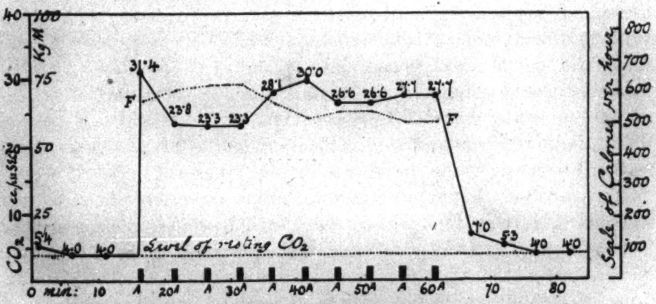
*Total work* = 6410 kgm. in 430 secs.
= 14·88 per sec.

*Average $CO_2$ discharge:*

| | | |
|---|---|---|
| During work | 26·87 c.c. per sec. | |
| At rest | 4·00 | ,, |
| Cost of work | 22·87. | |

*Mechanical Efficiency* (calculated on the assumption of a respiratory quotient = 0·84 giving 1 c.c. $CO_2$ = 2·5 kgms.) indicated by the dotted line F. F.

$$= \frac{14{\cdot}88}{22{\cdot}87 \times 2{\cdot}5} = 26 \text{ per cent.}$$

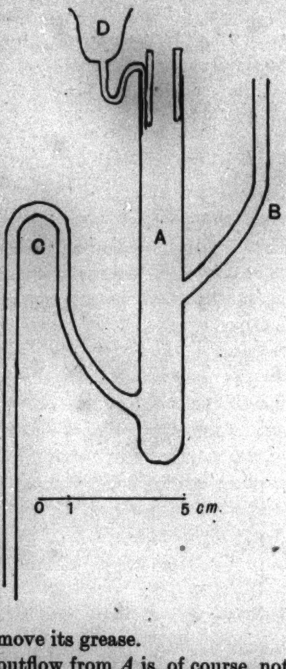


Cadet R. 64·1 kilos. Ten ascents of 20 metre staircase. (The dotted line F. F. indicates efficiency per cent.)

**A recording siphon.** By W. L. Symes.

This is a modification, made in one piece, and adapted for smaller volumes of fluid, of the pattern shown in 1916 (1) in which, as in Gunn's model, discharge is signalled by a Marey tambour. It is intended, primarily, for recording the output of the frog's heart.

It consists of a tube *A*, for the reception of the perfusate, joined to *B* for connection to the tambour, and to *C*. The lever of the tambour begins to rise when the accumulated fluid covers the lower orifice of *B* and signals discharge by an abrupt fall to its abscissa. The tubes *B* and *C* join *A* at an angle of about 60° and their junctions with *A* must be free from constriction. With a good heart, the siphon will discharge infallibly when the perfusate flows directly into *A*, but with a flagging heart, it may lose fluid by trickle. This is prevented by receiving the perfusate in the small detonating siphon *D* (1) which holds 0·2–0·3 c.c. and so hands on the output in volumes large enough to secure discharge of the recording siphon without (unrecorded) loss by trickle. The siphon *D* is held by a short rubber tube pressing it against the wall of *A*. If the outflow through *C* is too rapid the water column will break and lead to (variable) reflux into *A*. This may be prevented by choking, partially, the lower orifice of *C* with a feather previously washed in ether to remove its grease.



Any perfusate that enters *A* during outflow from *A* is, of course, not recorded. It is therefore necessary that the time between the commencement of outflow and the uncovering of the proximal orifice of *C* should be adequately brief. This time occupies 1–2 secs. in siphons of 5–10 c.c. capacity and, in conjunction with the temporary retention of the perfusate in *D*, ensures the emptying of *A*, without error, when the heart frequency does not greatly exceed 30 per minute.

The volume discharged by the siphon depends, in part, on the orientation of the latter. By rotating *A* clock-wise (about a horizontal axis perpendicular to the plane of the paper) the bend of *C* is raised and the capacity of the siphon is increased and vice versa. By this means the discharge of a siphon giving 8–12 c.c., when *A* is vertical, may be varied by ± 2–4 c.c. and adjusted, readily, to give any desired volume within these limits. The discharge of the siphon must, obviously, be adjusted and measured with the tambour connected to *B*.

### REFERENCE

(1) This *Journal*, **50**. 1915–16. These *Proceedings* p. xxxiii.

## A further demonstration of the part played by accessory food factors in the ætiology of rickets. By Edward Mellanby.

In a previous demonstration to this Society of puppies showing the part played by diet in producing and preventing Rickets, it was seen that, using a standard rachitic diet of bread and milk (175 c.c.), the disease was prevented by butter, cod liver oil and most other fats tested, increased milk (500 c.c.), meat and meat extracts, but was not prevented by yeast, linseed oil and the proteins of milk and meat. On the basis of these results, an attempt was made to improve the standard rachitic diet in order to produce a more rapid development of the disease and to render it less easy to prevent. In the dogs demonstrated today, the standard diet has been separated milk (175–350 c.c.), 70 per cent. wheaten bread *ad lib.*, linseed oil 5–10 c.c., yeast 5–10 grams, orange or lemon juice 3 c.c., sodium chloride 2 grams. This diet will produce a rapid development of rickets, easily recognisable by X-ray photograph within 6 weeks of beginning the diet. In the puppies exhibited it will be seen that

(1) lean meat (10 grams a day) only delays but does not prevent rickets and that the extent of the delay depends on the initial weight and rate of growth of the animal. The greater the rate of growth, the less is the effect of meat.

(2) increasing the separated milk to 350–400 c.c. per diem does not prevent rickets. This excludes the possibility that the calcium intake is deficient.

(3) 10 grams of butter or cod liver oil, even on the above diet, completely prevents rickets.

(4) 10 grams of cotton seed oil, olive oil or babassu oil per diem does not prevent rickets when substituted for linseed oil.

All vegetable oils are not equally ineffective in preventing rickets. Linseed oil is the worst of those tested, then in order of increasing merit, tentatively suggested, babassu, cotton-seed, peanut (arachis) and olive. A hydrogenated fat tested was similar to linseed oil. Suet and lard appear (experiment not completed) to prevent rickets if 10 grams of meat per diem is also eaten. A small amount of meat helps an otherwise ineffective fat.

The above facts are for the most part in keeping with the idea that rickets is a disease primarily due to a deficiency of Fat-soluble A. Animal fats are the best preventatives.

It is difficult, however, to fit the inhibitory action of lean meat, meat and malt extracts into this theory.

Other facts brought out in this work are:

(1) the more rapidly growing the dog, the more anti-rachitic factor is required to keep the growth straight.

(2) while not yet able to deny the necessity of Fat-soluble A being present in a diet to ensure growth, it is the case that many of my more rapidly growing puppies have had the least Fat-soluble A. This accessory factor seems to be more essential for ensuring normal growth as opposed to abnormal growth and keeping the tissues in good working order.

(3) taking this work in conjunction with my wife's research on factors affecting the formation of teeth[1], it appears that those substances containing and associated with Fat-soluble A are particularly concerned in the calcification processes of bones and teeth. Further, the close relationship between rickets and defective teeth (both as regards enamel formation and arrangement in the jaws) is placed on an experimental basis.

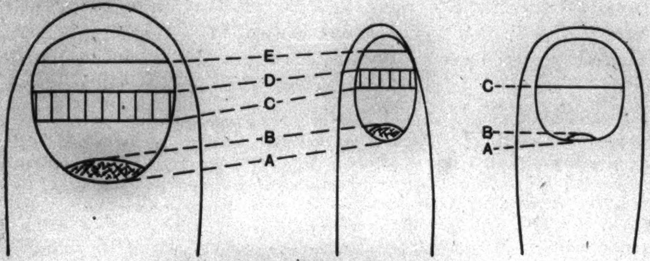
## A note in reference to segmental distribution of blood-vessels in the upper limb. By Judah Leon Jona.

It has occurred to me that possibly the following notes would be of interest. On 8. v. 1918 I stumbled, fell on my right elbow with arm out-stretched, and dislocated my right shoulder, the head of the humerus occupying a subclavicular position. It was reduced under chloroform within an hour of the occurrence. The X-ray photograph showed a little tearing up of the periosteum off the lower part of the neck of the humerus. The arm was put at rest—absolutely immobilised for 48 hours and then put up in a sling support which kept the shoulder joint and humerus practically immobilised and the fore-arm in flexion (the hand to the left breast) but free to pronate and supinate. The fingers were free. The arm was kept in this position until 31. v. 1918 (*i.e.* 24 days), being taken down morning and evening and moved gently for a few minutes. From 31. v. 1918 to 25. vi. 1918 (25 days) the arm was used, but not unnecessarily or vigorously. From 25. vi. 1918 onwards the arm was used freely.

---

[1] The effect of diet on teeth formation. May Mellanby, *Lancet*, December 7, 1918.

The appearance of grooves on the finger-nails of the hand of the affected arm attracted my attention and it is in regard to these that this communication is made. The grooves, well-marked, were confined to the nails of the thumb and index finger of the right hand, a doubtful mark appearing on the nail of the middle finger, the nails of all the other fingers of both hands being quite clear and free from grooves. The appearances and measurements on 7. viii. 1918, 26. viii. 1918 and 12. ix. 1918 were:



*E* was a thin narrow line, but well defined.
*CD* was a well defined broad groove.

7. viii. 1918.

| | Measurements | | |
|---|---|---|---|
| | Thumb | Index Finger | Middle Finger |
| *AB* | 0·25 cm. | 0·1 cm. | 0·05 cm. |
| *AC* | 0·4 | 0·4 | 0·4 |
| *AD* | 0·75 | 0·7 | |
| *AE* | 1·05 | 0·95 | |

26. viii. 1918.

| | |
|---|---|
| *AE* | 1·3 cm. |

12. ix. 1918.

| | |
|---|---|
| *AB* | 0·25 cm. |
| *AC* | 0·85 |
| *AD* | 1·2 |

**Rate of growth from 7. viii. 1918 to 26. viii. 1918 = 1 cm. in 80 days.**
**,, ,, 26. viii. 1918 to 12. ix. 1918 = 1 cm. in 90 days.**

From the date of the accident, 8. v. 1918, to the date of the first observation, 7. viii. 1918, were 90 days, which gives the position of the narrow line, *E*. The period of absolute rest was 24 days, which give a growth of nail of 0·3 cm., which corresponds to the space *ED*, and the period of use with the weakened arm from 31. v. 1918 to 25. vi. 1918, 25 days, gives the broad groove *CD*. It appears to me that in this phenomenon we have some segmental correlation between the innervation of the shoulder joint structures and the blood-vessels of the limb as evidenced in this case by alterations in the growth of the nails of two fingers, whereas all the fingers of the hand were, as far as it was possible to determine, under the same conditions.

# PROCEEDINGS
OF THE
# PHYSIOLOGICAL SOCIETY,
## *January* 25, 1919.

### **Modifications of Bielschowsky's method.** By C. Da Fano.

In 1914 I proposed a modification (*Atti Soc. Lomb. Scienze Med. Biol. Milano*, III. f. 4) of this method for the study of reticular tissue in spleen, lymph glands and other organs. An important point in the modification is the use of water redistilled on $KMnO_4$ spoken of in what follows as "redistilled water." This ensures elimination of any trace of organic matter, avoids the formation of troublesome precipitates and secures uniform staining. The 1914 method was briefly:

I. Fixation of small pieces of tissue in 10–20 % formalin, or in Kayserling's first fluid (48 hours at least). In a mixture of formalin and Müller's fluid, this must be reduced to 24–48 hours.—II. Washing in running tap water (24–30 hours), then in distilled water (24 hours).—III. Sections cut by freezing microtone 10–20$\mu$ thick, are washed in "redistilled water" (24 hours).—IV. Transfer sections to fresh "redistilled water" and then to a large volume of filtered 2 % $AgNO_3$ (prepared with "redistilled water") in a Petri dish taking care they do not touch each other. Here they are kept in the dark from 6 hours to 3 days.—V. Preparation of ammoniated $AgNO_3$ solution: to 5 c.c. of 20 % $AgNO_3$, prepared with "redistilled water," add 2 drops of 40 % NaOH and then ammonia, drop by drop, until the brown precipitate formed disappears; another drop is then added. Dilute to 40–70 c.c. with "redistilled water," and filter it through paper washed with the same water.—VI. Staining: the sections one by one are quickly washed in "redistilled water" and transferred to ammoniated $AgNO_3$ solution in known order; there they remain 20 minutes, and after another quick washing in "redistilled water" are placed in 20 % formalin (prepared with tap water and filtered) in the same order. After 3 or 4 sections are transferred, it is advisable to remove them into a fresh dish of 20 % formalin, and so on until all have reached this second bath where they remain 12 to 24 hours. Sections must be transferred by a glass rod, which is cleaned after every transference.—VII. Sections are toned with dilute (0·02 %) gold chloride solution, fixed with 5 % sodium hyposulphite, counterstained with lithium carmine, differentiated in acid alcohol, dehydrated in alcohols of increasing strength up to 95 %, placed in 95 % alcohol to which 1 drop per 30–40 c.c., of saturated alcoholic solution of picric acid has been added, then in fresh 95 % alcohol, cleared in carbol-xylol and mounted in balsam.

After such treatment the reticular tissue is black, large connective tissue bundles violet, nuclei pinkish with the largest chromatin granules black or brown, and the cytoplasm faint yellow; red corpuscles assume a pronounced yellow colour.

This modification with certain alterations may be used for the study of the finer structure of nerve cells. These alterations are as follow:

(1) Fixation in formalin must be prolonged for at least 3 weeks, or better still for 2 months. Though old material kept in formalin for years may give good results, this very prolonged treatment is apt to cause vacuolisation in the cells, especially in the superficial layer of the cortex cerebri. Attempts to obtain rapid (6–24 hours) fixation at 37° C. gave bad results.

(2) The sections may be placed after washing in "redistilled water" in anhydrous pyridine (6–12 hours), then repeatedly washed and left over night in "redistilled water" to get rid of all the pyridine. This treatment renders the neurofibrils a little sharper, but increases the length and cost of the method, and may cause precipitates to form especially where much myelin is present. In Bielschowsky's original method it was introduced between steps III and IV.

(3) It is possible to keep sections, which cannot be stained immediately, in "redistilled water," to which a few drops of formalin have been added, for some days or even a fortnight. Then thorough washing with "redistilled water" is imperative before they are transferred to the 2 % $AgNO_3$ solution.

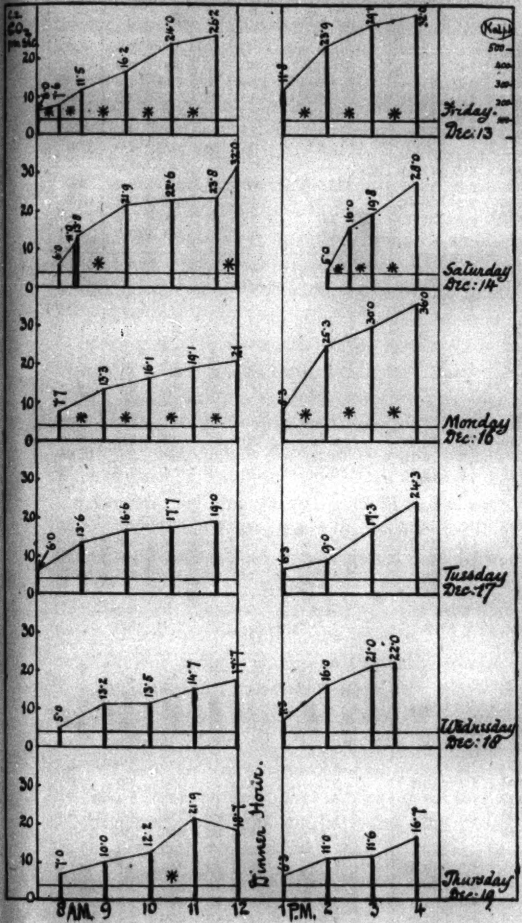
(4) Sections must not remain in the 2 % $AgNO_3$ solution more than 48 hours, or precipitates may form. The longer their stay there, the longer must be the washing before staining; this however must not exceed 5 minutes.

(5) The volume to which the ammoniacal $AgNO_3$ is diluted should be 35–45 c.c., and the sections remain in it 15–20 minutes. The subsequent washing before transferring the sections to 20 % formalin should not occupy more than 10–15 sec., and their stay in the final formalin solution (especially for cerebral cortex) should not exceed 2–3 hours. No counterstaining is necessary.

The method has given good results with all parts of the central nervous system of man and other mammals, except the spinal ganglia and cortes cerebelli; these latter tissues I am still investigating. The method is specially valuable for the demonstration of neurofibrils which are stained dark-grey to black with a slightly purplish tone.

## The $CO_2$ ordinate of a dock-labourer (Tom King) during six days work. (Winter hours 7.30 a.m. to 12; 1 p.m. to 4.30.)

By A. D. WALLER, M.D., F.R.S.



During the starred periods King was on piece-work (coaling at high pressure).

During the unstarred periods he was on time-work (miscellaneous) except on Saturday afternoon during which he worked overtime at relatively high pressure and not feeling well; he took a dinner interval of two hours on that day.

We notice that the piece-work of the first 3 days [which brings best money to the labourer] is more costly to him as indicated by $CO_2$, in comparison with the time-work of the last 3 days. Cf. especially the unstarred hours of Saturday morning with the starred hour of Thursday morning.

The increases ordinate during each morning and afternoon, and falls during the dinner hour and is higher at the end of the afternoon than at the end of the morning.

Averaging from the last 3 hours of the two complete piece-work days (Friday and Monday) and of the two complete time-work days (Tuesday and Wednesday), and allowing 4 c.c. per sec. as the resting $CO_2$, gives the following average values of the ordinate, i.e. of the net physiological cost:

*Piece-work* = 20·2 c.c. per sec.
*Time-work* = 13·4 „

At 5·91 cals per c.c. these amounts represent:

430 cals per hour
and 284 „ „

**Friday**, *Dec.* 13*th*, 1918.

| Time | Time | Litres | c.c. exp. air per sec. | °/. CO$_2$ | c.c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 7.30 a.m. | 60 | 20 | 333 | 1·8 | 5·99 |
| 8.0 | 50 | 19 | 380 | 2·0 | 7·60 |
| 8.30 | 45 | 20 | 444 | 2·6 | 11·54 |
| 9.30 | 40 | 23 | 575 | 3·0 | 16·25 |
| 10.30 | 30 | 24 | 800 | 3·0 | 24·0 |
| 11.30 | 35 | 23 | 657 | 4·0 | 26·2 |
| 1.0 p.m. | 60 | 22 | 366 | 3·2 | 11·71 |
| 2.0 | 35 | 24 | 685 | 3·5 | 23·97 |
| 3.0 | 30 | 23 | 766 | 3·8 | 29·10 |
| 3.30 | 30 | 24 | 800 | 4·0 | 32·0 |

**Saturday**, *Dec.* 14*th*, 1918.

| Time | Time | Litres | c.c. exp. air per sec. | °/. CO$_2$ | c.c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 8.0 a.m. | 60 | 18 | 300 | 2·0 | 6·0 |
| 8.30 | 30 | 15 | 500 | 2·2 | 11·0 |
| 8.30 | 30 | 16 | 533 | 2·6 | 13·8 |
| 9.30 | 30 | 22 | 733 | 3·0 | 21·9 |
| 10.30 | 30 | 20 | 666 | 3·4 | 22·6 |
| 11.30 | 30 | 21 | 700 | 3·4 | 23·8 |
| 12.0 | 30 | 24 | 800 | 4·0 | 32·0 |
| 2.0 p.m. | 60 | 15 | 250 | 2·0 | 5·0 |
| 2.30 | 40 | 20 | 500 | 3·2 | 16·0 |
| 3.0 | 35 | 21 | 600 | 3·3 | 19·8 |
| 4.0 | 30 | 24 | 800 | 3·5 | 28·0 |

**Monday**, *Dec.* 16*th*, 1918.

| Time | Time | Litres | c.c. exp. air per sec. | °/. CO$_2$ | c c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 8.0 a.m. | 60 | 21 | 350 | 2·2 | 7·70 |
| 9.0 | 30 | 20 | 666 | 2·0 | 13·32 |
| 10.0 | 30 | 21 | 700 | 2·3 | 16·10 |
| 11.0 | 30 | 23 | 766 | 2·5 | 19·15 |
| 11.45 | 30 | 21 | 700 | 3·0 | 21·0 |
| 1.0 p.m. | 60 | 20 | 333 | 2·5 | 8·32 |
| 2.0 | 30 | 19 | 633 | 4·0 | 25·32 |
| 3.0 | 30 | 21 | 700 | 4·4 | 30·0 |
| 4.0 | 30 | 24 | 800 | 4·5 | 36·0 |

**Tuesday**, *Dec.* 17*th*, 1918.

| Time | Time | Litres | c.c. exp. air per sec. | °/. CO$_2$ | c.c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 7.30 a.m. | 60 | 18 | 300 | 2·0 | 6·0 |
| 8.30 | 30 | 17 | 566 | 2·4 | 13·58 |
| 9.30 | 30 | 20 | 666 | 2·5 | 16·65 |
| 10.30 | 30 | 19 | 633 | 2·8 | 17·72 |
| 11.30 | 30 | 22 | 733 | 2·6 | 19·05 |
| 1.0 p.m. | 60 | 20 | 333 | 2·2 | 6·32 |
| 2.0 | 30 | 18 | 666 | 3·0 | 9·0 |
| 3.0 | 30 | 20 | 600 | 2·6 | 17·31 |
| 4.0 | 30 | 23 | 766 | 3·2 | 24·3 |

**Wednesday**, *Dec.* 18*th*, 1918.

| Time | Time | Litres | c.c. exp. air per sec. | °/. CO$_2$ | c.c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 8.0 a.m. | 60 | 15 | 250 | 2·0 | 5·0 |
| 9.0 | 30 | 18 | 600 | 2·2 | 13·2 |
| 10.0 | 30 | 15 | 500 | 2·7 | 13·5 |
| 11.0 | 30 | 17 | 566 | 2·5 | 14·1 |
| 11.45 | 30 | 19 | 633 | 2·8 | 17·7 |
| 1.0 p.m. | 60 | 20 | 333 | 2·2 | 7·32 |
| 2.0 | 30 | 15 | 500 | 3·2 | 16·0 |
| 3.0 | 30 | 18 | 600 | 3·5 | 21·0 |
| 3.45 | 30 | 18 | 600 | 3·8 | 22·0 |

**Thursday**, *Dec.* 19*th*, 1918.

| Time | Time | Litres | c.c. exp air per sec. | °/. CO$_2$ | c.c. CO$_2$ per sec. |
|---|---|---|---|---|---|
| 8.0 a.m. | 60 | 21 | 350 | 2·0 | 7·0 |
| 9.0 | 30 | 15 | 500 | 2·0 | 10·0 |
| 10.0 | 30 | 16 | 533 | 2·3 | 12·2 |
| 11.0 | 30 | 22 | 733 | 3·0 | 21·9 |
| 11.45 | 30 | 28 | 666 | 2·8 | 18·7 |
| 1.0 p.m. | 60 | 18 | 300 | 2·1 | 6·3 |
| 2.0 | 30 | 15 | 500 | 2·2 | 11·0 |
| 3.0 | 30 | 14 | 466 | 2·5 | 11·6 |
| 4.0 | 30 | 19 | 699 | 2·4 | 16·7 |

### A mechanical pipette for easy measurement of small volumes.

By H. C. Bazett.

This pipette has been devised for the rapid and accurate measurement of small amounts of fluids such as ·05 c.c., and has been used for taking samples of blood for making a 1 in 200 solution of hæmoglobin by means of a Du Boscq colorimeter. The tubes can be readily adapted for measuring other small volumes, and they save considerable time and labour in doing routine blood counts or Widal reactions.

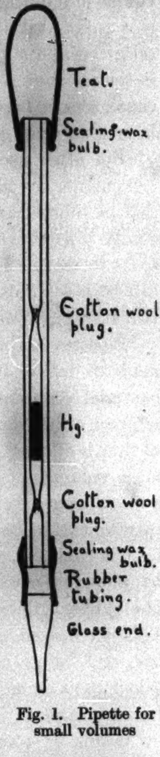


Fig. 1. Pipette for small volumes

The method depends on the use of a mercury valve, a modification of one used by Sir Almroth Wright. The passage of mercury through a capillary tube of fairly large bore is readily stopped by a small wad of cotton wool. The suction of a teat is limited by means of a valve consisting of mercury moving between two such obstructions, the resistance to air being slight and the movement therefore being quite rapid. For measuring small amounts such as ·05 c.c. for blood samples the pipette is made by heating a fine bore thick-walled glass tube so as to cause two constrictions of capillary bore. One of these constrictions is then plugged with a small wad of cotton wool and mercury is sucked up so as to fill the whole tube between the constrictions. The mercury is then discharged again into a watch glass and from it ·05 c.c. of mercury is deducted, the remainder being sucked up again into the pipette. The other capillary end is then plugged with cotton wool and it is evident that a teat can now only move the mercury through a length of the tube corresponding to a volume of ·05 c.c. In this way the valve is formed and the teat and some small glass ends connected to the valve by rubber tubing are all that are required further.

In actual practice it will be found that, owing to the expansibility of the air, such a pipette will take up rather less than ·05 c.c. so that each pipette must be standardised. This can be easily done; small amounts of mercury are deducted until the pipette delivers the required amount. Glass ends of roughly the same size and shape must be used in taking samples, and a number of these may be kept at hand. In taking blood samples the blood is sucked up into these from a paraffined watch glass, and is discharged into a test tube of water or saline, the glass end carrying the blood being dropped also into the test tube. Care has to be taken to leave the pipette a couple of minutes after handling the lower end before it is used to take a sample, as the instrument is very sensitive and is much affected by the small changes in temperature resulting from handling. If these precautions are taken, I have found it very accurate, having an error of only ± ·25 %.

For other uses, such as the taking of blood for cell counts, the blood may be taken in the same way and be diluted as required. For white cell counts a similar pipette can be easily made to discharge ·45 c.c. so as to make a 1 in 10 dilution; in fact, any dilution can be made automatically as required by the use of two such pipettes.

Pipettes acting on the same principle may be made to deliver 15, 10, 9, 8 and 5 drops of saline or bacterial emulsion for Widal tests and if a large number of bloods have to be examined a great saving of time is effected. In repeated use for comparatively large volumes of fluid the mercury becomes damp and no longer works well, so that for such use the pipette has to be modified to allow the mercury to be changed and renewed every day. This is done by plugging one capillary end by a small cap of fine rubber tubing carrying a wad of cotton wool. This is removed and the mercury is discharged after use, and before using again fresh clean mercury is taken up as far as a graduation mark on the pipette. In such sizes it is easiest to fit on the glass end by means of a small rubber cork instead of with rubber tubing.

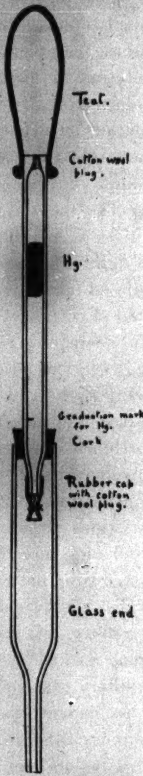


Fig. 2. Pipette for larger volumes

## **Some physiological effects of anoxæmia.** By C. H. Kellaway.

This investigation was undertaken with the object of throwing light on the physiological changes which occur as the result of reduced tensions of oxygen, as at high altitudes.

The following changes have been and still are under observation:

Apart from the direct action on the central nervous system of insufficient oxygenation of the blood there are other changes which might be supposed to play a part in the complex of symptoms produced by anoxæmia.

### 1. *The effect on the efficiency of the circulation.*

There is no evidence that even prolonged exposure, 3 to 4 hours, to percentages of oxygen equivalent to those existing at heights of from 19,000 to 23,000 feet produces any notable change in the efficiency of the circulation in the cat. At the end of such a period the animal was anæsthetised with ether, while still being supplied with the same mixture of low oxygen content, and the arterial blood-pressure was found to be well sustained at about 160 mm. Hg.

### 2. *Effect on the alkali reserve of the plasma.*

Experiments have been made on the cat and goat. Immediately after exposure for 1½ hours to an atmosphere containing about 7 per cent. of oxygen the plasma of a cat showed only a trifling diminution in alkali reserve, the $CO_2$ capacity falling from 34·9 to 33·6 per cent. Two hours after return to normal air, however, a further and more considerable fall took place, to 29 per cent. $CO_2$ capacity. During the next hour the alkali reserve recovered to the original level.

In the goat a similar phenomenon was observed. The exposure was for 25 minutes to 11 per cent. $O_2$ and for a further period of 55 minutes to 8·5 per cent. $O_2$. The $CO_2$ capacities of the plasma were 64 per cent. before exposure, 62 per cent. at the end of the period of anoxæmia, and 58 per cent. after a further period of 1 hour under normal conditions.

### 3. *The effect on the output of adrenalin.*

This has been studied by noting the effect on the cat's pupil, sensitised by previous removal of the superior cervical ganglion on one side.

I find that the paradoxical dilatation of such a pupil with asphyxia is entirely the result of deficiency of oxygen. If the cat is given air containing little oxygen or pure nitrogen to breathe for a short period a pronounced dilatation of the denervated pupil occurs, which may be detected for about 10 minutes or sometimes longer after the breathing of air has been resumed.

On the contrary, dyspnœa produced by breathing air containing 10 per cent. of $CO_2$ produces no perceptible reaction of the pupil, and even 5 minutes of 20 per cent. $CO_2$ causes only such trivial and evanescent dilatation as is probably referable to the associated nervous excitement.

In one cat after section of both splanchnics some weeks previously a very slight reaction of the pupil was caused by anoxæmia (5 minutes 6 per cent. $O_2$). Later, after removal of both suprarenal glands, a similar anoxæmia caused no reaction of the pupil.

In another cat the administration of nitrogen for one minute produced maximal dilatation of the sensitised pupil. A similar exposure after the suprarenals had been removed produced a definite but very much smaller effect.

It seems clear that the paradoxical reaction of the pupil to asphyxia is wholly due to anoxæmia and mainly to stimulation of the central nervous system by want of oxygen producing through the splanchnics an accelerated secretion from the adrenal glands. Such an accelerated output of adrenalin can be detected as the result of exposure to atmospheres containing 10 per cent. or even 12 per cent. of oxygen for brief periods.

### 4. *Hyperglycœmia.*

The well-known hyperglycæmia of asphyxia is likewise wholly an effect of anoxæmia. Observations have been made on the rabbit, cat and goat. In all of them I find a large rise of blood sugar as a result of anoxæmia. In the cat a similar hyperglycæmia was produced by short periods of deficient oxygen, after section of the splanchnics and even after removal of both suprarenal glands.

While, therefore, the accelerated output of adrenalin must be supposed to participate in the effect, the rise in blood sugar must be in the main due to a direct action of the anoxæmia upon the liver.

Administration of excess of $CO_2$ has never in my experience given rise to even the slightest increase in blood sugar.

All operations in which the animal recovered after anæsthesia were performed by Dr H. H. Dale.

## **The relation between respiration and the pulse rate.** By F. A. Bainbridge and R. Hilton.

That a close correspondence usually (though not invariably) exists in normal individuals between the pulmonary ventilation and the pulse rate is well known; and this relationship, particularly when the activity of the respiratory centre is increased, has been ascribed, at least in part, to an irradiation or overflow of impulses from the respiratory to the vagus centre. In the following experiments an attempt has been made to demonstrate the presence or absence of such irradiation. Two methods have been employed, the experiments being made on cats fully anæsthetised with ether alone or with urethane and ether.

In one method, the spinal cord was divided at the level of the 1st cervical vertebra and the respiratory movements of the alae nasi or mylohyoid muscle were directly observed. In the other, the chest was opened, the phrenic and the cardiac accelerator nerves were divided and a graphic record was taken of the respiratory movements of the chest wall. Artificial respiration was maintained by means of a pump. Stoppage of the pump, although followed either at once or after a brief interval by vigorous respiratory effects, led to no change in the pulse rate (Fig. 1) until with the onset of asphyxia slowing of the pulse took place. In other experiments, the animal was made to breathe almost pure oxygen, the blast of the pump being reduced so as to cause very slight inflation of the lungs. In these circumstances the oxygenation of the blood was adequate, but the rising tension of carbonic acid in the blood evoked vigorous respiratory efforts; the pulse rate remained unaltered for some time and then rapidly decreased. The presence of vagus tone, and therefore the possibility of acceleration, was always demonstrated either by cutting both vagi towards the end of the experiment and noting the increase in rate or by bringing about acceleration of the pulse by other means during the experiment. In one experiment, in which the chest had been opened, the pulse rate was 114, when the artificial respiration was stopped, and although the animal made vigorous respiratory efforts, the pulse rate only rose to 116. On subsequent section of the vagi, the pulse rate rose to 180.

These experiments furnish no evidence, therefore, that the tone of the vagus centre can be altered by irradiation or overflow of impulses from the respiratory centre. Nor do they suggest that the vagus centre is directly excited by a slight rise in the $C_H$ of the blood, although, as

Hill and Flack[1] showed, a marked rise in the $C_H$ of the blood slows the heart by increasing vagal tone.

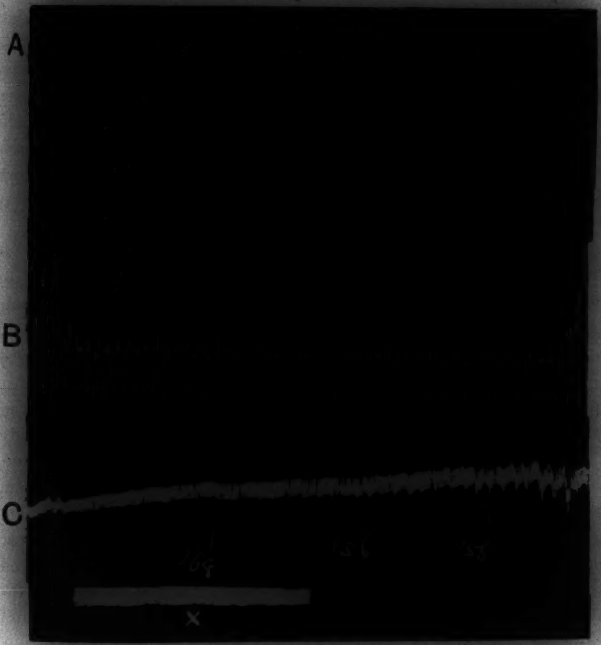


Fig. 1. The figures represent the pulse rate.
A = Respiratory efforts. B = Blast of pump.
C = Blood-pressure (98 mm. at outset).
During the period X pure oxygen was breathed.

---

[1] Hill and Flack. This *Journal*, **37**. p. 77. 1908.

# PROCEEDINGS

OF THE

# PHYSIOLOGICAL SOCIETY,

*February* 22, 1919.

## The time-relation between muscular work and $CO_2$ discharge.

By A. D. Waller.

In taking the $CO_2$ ordinate of dock labourers during their day's work it is necessary to cause the least possible loss of time. In preference to a mask and bag requiring adjustment, I have used a comparatively small (20 litre) india-rubber pillow fitted with a valve and a two-way tap by which the beginning and end of sampling can be sharply established with a stop-watch. The subject breathes by mouth with a clip on his nose for one or preferably half-a-minute, holding the bag himself during this brief interruption of work.

Strictly speaking, therefore, the sample is taken not during but immediately after the actual work in progress.

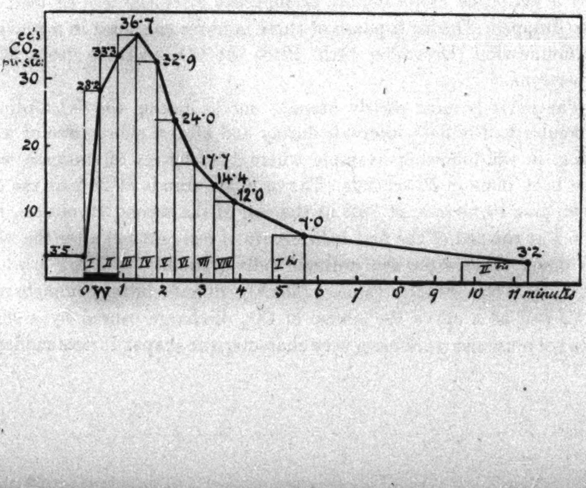
The object of this communication is to justify the procedure and to show the importance for fair sampling of taking the sample for as brief a period as half-a-minute *immediately* after the tool or load has been dropped. During a pause of three minutes as shown in a previous communication (December 14th, 1918) the $CO_2$ ordinate drops 30 to 40 per cent.

The point is most clearly brought out by taking the $CO_2$ ordinate at regular half-minute intervals during and after a short piece of work as e.g. in the following example where 1410 kg-ms of staircase work have been done in 52 seconds. The ordinate stands at 28·2 at the end of the first 10 metres, at 33·3 at the end of the second 10 metres, and at 36·7 at the end of the first half-minute of rest (sitting) after the work was done. Thereafter the ordinate falls gradually to 3·2, 3·5, which last figure is the ordinary value of the $CO_2$ ordinate during complete rest.

Viewed as a curve the course of $CO_2$ discharge caused by a short period of muscular work has a very characteristic shape. It rises suddenly (by increased $CO_2$ percentage as well as by increased ventilation when no doubt a pump effect must be taken into account) and comes to a maximum during the first half-minute of repose. It declines more gradually to the normal level of quiescence, which in the case of a healthy subject is reached in about 10 minutes. The rate of this decline varies however with the health, age and fitness of the worker as will be shown in further communications.

Feb. 9. Cadet TAYLOR. Age 19. Weight 70·5 Kilos. The $CO_2$ ordinate before, during and after a short period of work (ascent of a 20-metre staircase in 52 seconds).

| Time | | No. of sample | Time of sample, | Ventilation | | $CO_2$ | | Conditions | |
|---|---|---|---|---|---|---|---|---|---|
| min. | secs. | | secs. | Litres | c.c.'s per sec. | % | c.c.'s per sec. | | |
| 0 | 0 | | 100 | 10 | 100 | 3·5 | 3·5 | At rest (sitting) | |
| 10 | 0 | | 100 | 10 | 100 | 3·5 | 3·5 | ,, ,, | |
| 10 | 25 | I | 25 | 16 | 640 | 4·4 | 28·2 | Ascent of 1st ten metres | Work 1410 kg-ms |
| — | 52 | II | 27 | 20 | 740 | 4·5 | 33·3 | Ascent of 2nd ten metres | |
| 11 | 25 | III | 30 | 22 | 733 | 5·0 | 36·7 | Sitting. (3 secs. interval for change of expiration bag) | |
| 12 | 0 | IV | 30 | 21 | 700 | 4·7 | 32·9 | Walked 12 metres. (5 secs. interval for change) | |
| 12 | 30 | V | 30 | 15 | 600 | 4·0 | 24·0 | At rest (reading) | |
| 13 | 0 | VI | 30 | 14 | 467 | 3·8 | 17·7 | | |
| 13 | 30 | VII | 30 | 12 | 400 | 3·6 | 14·4 | | |
| 14 | 0 | VIII | 50 | 10 | 333 | 3·6 | 12·0 | | |
| 15 | 30 | I (*bis*) | 50 | 10 | 200 | 3·5 | 7·0 | | |
| 21 | 20 | II (*bis*) | 100 | 9 | 90 | 3·5 | 3·2 | | |
| 30 | 0 | III (*b·s*) | 100 | 10 | 100 | 3·5 | 3·5 | | |



**Metabolism and cooling power.** By LEONARD HILL and D. HARGOOD-ASH.

The influence of temperature on metabolism has been studied in the still air of calorimeters where adaptation of the sense organs takes place.

In the metabolism experiments discussed below the Douglas bag method was used for determining the respiratory exchange in the open air, and the caloric output determined from the oxygen values by the Zuntz method of calculation. The experiments were carried out to determine:

(1) how far a relation exists between the heat loss of the dry kata thermometer and the heat loss of the body,

(2) the variation of metabolism due to changes of atmospheric conditions in the open air,

(3) to what extent the metabolism is influenced by clothing.

In all the experiments observations were taken of wet and dry bulb temperatures, wet and dry cooling powers by the kata, and the cheek temperature.

(1) The heat loss $H$ (expressed in millicalories per sq. cm. per sec.) is given by the formula $H=(\cdot 27+\cdot 49\sqrt{v})\theta$, where $\theta=\phi-t$, $\phi$ being the mean temperature of the range of cooling, $t$ the centigrade temperature of the surrounding air and $v$ the wind velocity in metres per second.

The results show that no constant relation exists between the heat loss of the kata and that of the body, but that if $\phi_1$ be the cheek temperature, and the cooling power $H_1$, with $\phi_1$ as the mean temperature of the range of cooling, be calculated from the formula

$$H_1=H\frac{(\phi_1-t)}{(\phi-t)}=H\frac{\theta_1}{\theta},$$

deduced from that given above, then for outdoor conditions the ratio of the heat loss of the kata to that of the body expressed in millicalories per sq. cm. per sec. is from 6 to 7. This relation evidently does not hold when the value of $H$ is less than 6.

Table I gives some of the figures obtained and shows how the ratio decreased when the observations were taken in a heated atmosphere, both in a conservatory and on a hot day out of doors.

(2) Table I also indicates how the metabolism varies with temperature and cooling power. The variation takes place in the absence of shivering.

(3) Table II shows the difference in metabolism caused by variation of clothing. In experiment (A) the conditions were a snowstorm,

the clothing was flannel vest and shirt, waistcoat, coat, overcoat, gloves, muffler, hat and the feet were on a hot bottle, and in (B) overcoat, gloves, muffler, hat and hot bottle were removed, and coat and waistcoat thrown open, causing an increase in the heat loss of 62·6 % or taking the heat loss on a warm day as 1, a total increase of 87 %. In experiment (C) a thin vest, cotton blouse, two coats and muffler were worn, and in (D) the coats and muffler removed, the increase of heat loss being 76·3 %, or the total increase over that on a warm day of 160 %.

This table also shows that the ratio of heat loss of kata to that of the body given above as 6—7 does not hold in the case of very light clothing, experiment (D), or very warm clothing, experiments (A) and (C). That this is to be expected may be seen by writing the ratio

$$\frac{H_1}{\text{Heat loss}} = \frac{H}{\theta} \cdot \frac{\theta_1}{\text{Heat loss}} = \text{constant},$$

or since $H/\theta$ is a constant for any given conditions, we have $\theta_1$/Heat-loss = constant, and as is shown above the heat loss will tend to increase with thin clothing on a cold day while the cheek temperature and therefore $\theta_1$ will tend to decrease.

TABLE I.

| Date | Subject | Air temp. | Cheek temp. | $H$ | $H_1$ | Heat loss of body | $\frac{H}{\text{Heat loss}}$ | $\frac{H_1}{\text{Heat loss}}$ |
|---|---|---|---|---|---|---|---|---|
| 4. vi. 18 | L.H. | 14·5° C. | 31·0° C. | 10·5 | 7·9 | 1·32 | 8·0 | 6·0 |
| 3. vi. 18 | ” | 17·3 | 31·5 | 11·9 | 8·8 | 1·29 | 9·2 | 6·7 |
| 30. v. 18 | ” | 18·7 | 33·5 | 8·0 | 6·6 | 1·03 | 7·6 | 6·3 |
| 5. x. 18 | F. | 11·5 | 26·0 | 13·4 | 7·8 | 1·27 | 10·5 | 6·1 |
| 4. x. 18 | ” | 13·6 | 27·0 | 12·4 | 7·4 | 1·24 | 10·4 | 6·2 |
| 2. x. 18 | ” | 12·5 | 27·5 | 13·7 | 8·3 | 1·04 | 11·0 | 6·7 |
| 22. vii. 18 | P.H. | 17·6 | 29·0 | 11·2 | 6·7 | 1·03 | 10·8 | 6·5 |
| 10. x. 18 | ” | 14·9 | 24·7 | 13·7 | 6·2 | 1·20 | 11·4 | 5·2 |
| 11. iii. 18 | H.-A. | 13·5 | 27·5 | 11·4 | 6·9 | 1·04 | 10·9 | 6·7 |
| 14. iii. 18 | ” | 15·0 | 22·0 | 21·0 | 6·8 | 1·14 | 18·4 | 6·0 |
| 9. iv. 18 | ” | 6·2 | 21·5 | 16·1 | 8·1 | 1·28 | 12·6 | 6·3 |
| 8. iii. 18 | ” | 2·0 | 16·0 | 20·2 | 8·2 | 1·39 | 14·5 | 5·9 |
| 17. iv. 18 | ” | 6·0 | 19·0 | 21·9 | 9·3 | 1·49 | 14·7 | 6·3 |
| 18. iv. 18 | ” | 6·2 | 19·5 | 21·9 | 9·6 | 1·52 | 14·4 | 6·3 |
| 19. iv. 18 | ” | 3·0 | 15·5 | 30·4 | 11·3 | 1·78 | 17·1 | 6·4 |
| 5. vi. 18 | L.H. | 25·0 | 35·0 | 2·8 | 2·4 | 1·31 | 2·1 | 1·3* |
| 23. iii. 18 | H.-A. | 21·5 | 36·0 | 2·8 | 2·7 | 1·20 | 2·3 | 2·3* |
| 22. v. 18 | ” | 26·7 | 35·0 | 5·0 | 4·6 | 1·25 | 4·0 | 3·7** |
| 21. v. 18 | ” | 27·0 | 36·0 | 5·3 | 5·2 | 1·23 | 4·3 | 4·2** |

* In conservatory. ** Out of doors, warm day.

TABLE II.

| Date | Subject | Air temp. | Cheek temp. | $H$ | $H_1$ | Heat loss | $\frac{H_1}{\text{Heat loss}}$ |
|---|---|---|---|---|---|---|---|
| 5. ii. 19 | L.H. (A) | 1·0° C. | 20·0° C. | 23·5 | 12·6 | 1·15 | 10·9 |
| " | " (B) | 0·4 | 18·5 | 27·7 | 13·9 | 1·87 | 7·3 |
| 31. i. 19 | H.-A. (C) | −2·5 | 20·0 | 19·7 | 11·4 | 1·48 | 7·7 |
| " | " (D) | −2·5 | 17·5 | 20·0 | 10·3 | 2·61 | 3·9 |

Our results are given in millicalories per sq. cm. per sec.
Multiply by 36 to convert into k. cal. per sq. m. per hour.

# PROCEEDINGS
OF THE
# PHYSIOLOGICAL SOCIETY,
## *March* 22, 1919.

### **"Work" measured by $CO_2$.** By A. D. WALLER, M.D., F.R.S.

In contribution to our discussion concerning labour requirements as gauged by indirect calorimetry, I wish to lay stress on the importance of recognising the practical value of observing the $CO_2$ discharge alone. As already urged in previous communications to the Society, an expeditious method, involving an error of ± 5 per cent., affords a useful view of the physiological cost of work in terms of the varying $CO_2$ discharge, and should be admitted to rank as a valuable adjunct to the more elaborate method practised in the Carnegie Laboratory by Benedict and his coadjutors by which observations with an error of ± ·1 % can be made. In many cases the simple observation of $CO_2$ is alone possible, the multiplication of data obtainable by a ± 5 % method may be expected to afford as accurate a final result as that afforded by the fewer results obtainable by the ± ·1 % method. Moreover the numerous readings obtainable by the former method can of course be checked if desired by occasional measurements of oxygen and the computation of energy-value in accordance with the value of the R.Q. accurately observed and computed. Failing this last proviso the results of energy calculations from simple $CO_2$ data on the assumption that the R.Q. has been of average value (0·85) during observation, is likely to afford the more accurate results in any hands but those of a highly trained and expert gas-analyst.

The range of error incurred by the assumption of an average R.Q. = 0·85 is shown in the following table. An error of ± 0·05 in the R.Q. implies an error of less than ± 5 % in the energy value of $CO_2$.

*Equivalents of* 1 *c.c.* $CO_2$.

| R.Q. | Calories | Kgms. |
|---|---|---|
| 1·00 | 5·047 | 2·155 |
| 0·95 | 5·317 | 2·270 |
| 0·90 | 5·587 | 2·386 |
| **0·85** | 5·856 | **2·501** |
| 0·80 | 6·126 | 2·615 |
| 0·75 | 6·396 | 2·731 |
| 0·70 | 6·666 | 2·846 |

## SUMMARY OF OBSERVATIONS.

| | | | I | II | III | IV | V |
|---|---|---|---|---|---|---|---|
| | | Nature of work | Kg-m. per sec. | $CO_2$ c.c. per sec. net | $\frac{\text{Kg-m.}}{CO_2}$ | Eff. % | Cals. per hour |
| **A.** | | | | | | | |
| 1. | A. D. W. 63 y., 83 kos. | Ascent of 20 metres | 30·0 | **38·0** | 0·79 | 31·6 | — |
| 2. | J. C. W. 27 y., 73·8 kos. | ,, ,, | 27·3 | **27·6** | 0·99 | 39·6 | — |
| 3. | J. A. G. 98 kos. ... | ,, ,, | 43·5 | **38·5** | 1·13 | 45·0 | — |
| 4. | W. L. S. 63·4 kos. ... | ,, ,, | 13·6 | **21·5** | 0·63 | 25·0 | — |
| 5. | M. G. 38½ y., 54·5 kos. | ,, ,, | 18·2 | **22·5** | 0·81 | 32·4 | — |
| 6. | M. S. P. 50 y., 70 kos. | ,, ,, | 17·5 | **28·0** | 0·62 | 25·0 | — |
| 7. | E. H. S. 63 kos. ... | ,, ,, | 13·7 | **18·8** | 0·73 | 29·2 | — |
| 8. | G. De D. 25 y., 57 kos. | ,, ,, | 19·0 | **24·0** | 0·79 | 31·6 | — |
| 9. | Cadet R. 18½ y., 64 kos. | ,, ,, | 14·9 | **22·9** | 0·65 | 26·0 | — |
| 10. | Cadet B. 19 y., 69 kos. | ,, ,, | 20·3 | **22·5** | 0·91 | 36·4 | — |
| 11. | Cadet T. 19 y., 70·5 kos. | ,, ,, | 27·1 | **33·2** | 0·82 | 32·8 | — |
| 12. | J. Perry 51 y., 83 kos. | ,, ,, | 21·6 | **20·8** | 1·04 | 41·6 | — |
| **B.** | | | | | | | |
| 12. | J. Perry ... ... | Dock labour (time work) | — | **12·0** | — | — | 253 |
| 13. | T. King ... ... | ,, ,, | — | **13·5** | — | — | 285 |
| 13. | ,, ... ... | Coaling (piece work) | — | **21·0** | — | — | 443 |
| 1. | A. D. W. ... ... | Walking, 3·6 m.p.h. | — | **20·0** | — | — | 422 |
| 2. | J. C. W. ... ... | "Marking time" (120 per min.) | — | **11·2** | — | — | 236 |
| 2. | ,, ... ... | Walking, 3·4 m.p.h. | — | **11·0** | — | — | 232 |
| 2. | ,, ... ... | ,, 3·9 ,, | — | **17·2** | — | — | 362 |
| 2. | ,, ... ... | Running, 5·9 ,, | — | **29·7** | — | — | 626 |
| 2. | ,, ... ... | ,, 7·2 ,, | — | **51·8** | — | — | 1091 |
| 7. | E. H. S. ... ... | Walking, 4·0 ,, | — | **16·0** | — | — | 337 |

The first part **A** of the Table gives the cost (in c.c.'s $CO_2$ per sec.) of known work (in kg-ms per sec.). The second part **B** gives the cost in $CO_2$ of work of unknown mechanical value.

Col. I gives rates of work, which consisted in one or more ascents of a 20 metre staircase in "go as you please" time measured in seconds by stopwatch. Each number represents the quotient

$$\frac{\text{weight in kilos} \times 20 \text{ metres}}{\text{time in seconds}} = \text{kg-ms per sec.}$$

Col. II (in block figures) gives the amounts of $CO_2$ per sec. during the 30 secs. *immediately* after each ascent. (The resting $CO_2$, between 3 and 4 c.c. per sec. different subjects has been subtracted from the total $CO_2$.)

Col. III the $\frac{\text{work}}{\text{cost}}$ or $\frac{\text{kg-m.}}{CO_2}$ quotients, i.e. the work in kg-m. done per 1 c.c. $CO_2$.

Col. IV the efficiency per cent. by converting $CO_2$ into its mechanical equivalent at the R.Q. $=0·85$, i.e. $\frac{\text{kg-m.}}{2·5\ CO_2} \times 100$ or $40\,\frac{\text{kg-m.}}{CO_2}$.

Col. V. Calories per hour corresponding with the $CO_2$ per sec. of Col. II at R.Q. $=0·85$.

I have not thought necessary to work out these calorie values for the first part of the table, nor to compute the mechanical values of $CO_2$ in part **B** theoretically obtainable from $\frac{\text{kg-m.}}{CO_2}$ values given in part **A**. Detailed measurements of subjects 9, 12 and 13 have been given in previous communications (*Proceedings* Dec. 14 and Jan. 25).

## **Energy exchange in man.** By AUGUST KROGH.

Prof. Lindhard and I have during the last two years made a large number of experiments (over 200 determinations upon six subjects) on the energy requirements of muscular work on the bicycle ergometer with the object of studying the influence of the kind of substance katabolized (as expressed by the R.Q.) on the efficiency of the work.

Our experiments have been done by means of a Jaquet chamber and the outgoing and ingoing air was analyzed on a new gas analysis apparatus accurate both for $O_2$ and $CO_2$ to about $\frac{1}{1000}$ per cent. All the determinations on each subject have been made with a constant amount of work throughout. The amount of technical work performed varied for the different subjects between 0·7 and 1·1 cal. per minute. The metabolic energy (exclusive of the metabolism during rest) corresponding to 1 cal. of technical work varied with the subjects between 5·3 and 4·2 cal. (R.Q. = ·85) corresponding to efficiences between 19 and 24 %.

Even in this simple kind of work to which the subjects were accustomed a distinct influence of training upon the efficiency could be observed from about two hours of work per day over 15–25 days. In one case the metabolism per cal. decreased during 25 days from 4·6 to 4·3 cal. (R.Q. = ·85).

We found further that the metabolism per cal. technical work is on the whole a straight line function of the quotient, that is of the amount of fat katabolized, and distinctly higher for fat than for carbohydrate. The effect of protein has not been studied and the quantity of protein in the diet was kept at a low figure throughout.

When fat is utilized for muscular work a loss of energy takes place amounting according to our experiments to 8–12 per cent. of the fat katabolized.

This result can, we believe, be most naturally explained on the hypothesis, for which strong evidence has been brought forward by Hopkins, Fletcher, Hill and their collaborators, that carbohydrates can be utilized more or less directly by the muscles, while fat must first be converted to some substance allied to carbohydrates.

Most of our subjects had a very distinct feeling that the work was easier to perform on a carbohydrate diet than on any other and became very tired when working on a fat diet. Two, however, failed to observe any appreciable difference in this respect.